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Volume 23 Number 2

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STUDIES IN VASCULAR REPAIR

V. Experiments with the Use of Free Venous Transplants for Bridging Aortal Defects*

HARRIS B. SHUMACKER, JR., GERD SCHLOSS, LESLIE W. FREEMAN, EDWARD E. WAYSON, AND NICHOLAS H. STAHL

The expanding clinical application of techniques of vascular surgery has made desirable a better understanding of the functional and histological fate of various types of free vascular transplants used for bridging arterial defects. Though considerable useful information has been recorded by earlier investigators,[16] it was hoped that a study of fresh, preserved and fixed vein grafts employed as transplants to the thoracic aorta might prove fruitful.

Materials and methods

Adult and young adult mongrel dogs were used, and in one instance a young puppy (No. 705). In those experiments with autografts a segment of the distal end of the inferior vena cava was removed aseptically through a muscle-splitting right lower quadrant incision. The excised specimen was placed in physiological saline solution until the thoracic aorta could be exposed and prepared for its reception. In the case of fresh homografts a segment of the distal inferior vena cava was similarly removed from one animal while the thoracic aorta of another animal was being exposed by a second operator. Veins preserved by freezing were obtained in the same manner and were immediately quick-frozen by placing them in sterile glass tubes which were immersed in a mixture of solid carbon dioxide and ether. After the veins were thus frozen the tubes were properly labeled and stored in a deep freeze unit maintained at a temperature of minus 3° C. or less. Other segments of vena cava were fixed in 10% formalin solution as soon as they were obtained. In two dogs, formalin-fixed innominate veins of dogs were used as transplants, and in two others, fixed human iliac veins. The frozen veins were thawed in physiological saline solution at room temperature immediately before their use for transplantation. The formalinized grafts were washed thoroughly in physiological saline solution before being used as grafts.

All operations were performed under intravenously administered Nembutal anesthesia. Rhythmic inflation of the lungs with air was maintained during operation by a special machine connected with an intratracheal tube. With the animal placed on the right side a left thoracotomy was carried out, generally through the fourth interspace with posterior transection of the third or third and fourth ribs. Occasionally the chest was entered after resection of a long segment of the fourth rib. The mediastinal pleura was opened over the upper part of the descending aorta which was freed from its first portion downward for a distance of about 2½ cm. This usually necessitated division of two or three pairs of intercostal arteries. The aorta was then sharply transected between special non-crushing aorta clamps after removing the gross adventitia. The

* From the Departments of Surgery and Pathology, Yale University School of Medicine, and the Department of Surgery, the Indiana University Medical Center, Indianapolis, Indiana. Aided by a contract with the Office of Naval Research, the United States Navy.

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vein graft was then interposed, using continuous everting mattress sutures of number 5 Deknatel for the anastomosis. In one animal (No. 705) the anastomosis was accomplished with interrupted everting sutures. The distal clamp was then released and if any significant leak was noted, it was closed with an additional suture. The proximal clamp was next released, the mediastinal pleura sutured over the aorta and graft, the lungs re-expanded, and the chest wall closed in layers with interrupted silk sutures.

Though the anastomoses were all accomplished in a good technical manner, they were done with varying rapidity, generally proportionate to the operator's personal experience with vascular suture techniques. As a rule, no difficulties ensued provided the transplantation was accomplished rapidly enough so that the aorta was occluded for half an hour or less. In general, when the aorta was occluded for longer periods a spastic paraplegia ensued. In two animals, dogs 639 and 435, the paralysis was partial and temporary; in five, dogs 421, 516, 552, 614, and 657, it was complete and permanent. Neither antibiotics or anticoagulants were administered. The animals were maintained on a standard kennel diet. They were sacrificed at varying intervals. A few animals died shortly after operation. Some died later, most commonly of pulmonary infection.

In some instances aortograms were made before the animals were sacrificed. These were obtained by exposure of an x-ray film just at the completion of the rapid injection of from 8 to 25 cc. of 70% Diodrast into the external jugular vein; the dogs were anesthetized with sodium pentothal for these procedures and were placed in the lateral position. In some cases simultaneous Hamilton manometer recordings of carotid and femoral blood pressures were made before the dogs were sacrificed.

In each case the heart and thoracic aorta were carefully exposed and removed as soon after the death of the animal as was possible. In many of them colored photographs of the intact and opened specimen were obtained. The segment of the transplant and the adjoining proximal and distal aorta was then fixed in Zenker formol solution. Some were embedded in paraffin, others in celloidin. The cut sections were stained (1) with hematoxylin and eosin, (2) with Masson's trichrome stain, (3) with Verhoeff's elastic stain combined with either Van Gieson's stain or Masson's trichrome stain, and (4) with Mallory's phosphotungstic acid hematoxylin.

Normal histology of the inferior vena cava

The intima of the inferior vena cava consists only of the endothelium. The media is rather narrow and varies considerably in different dogs due to the varying distribution of smooth muscle cells and connective tissue. In some it is composed of an inner layer of circularly arranged smooth muscle cells separated by thin collagenous fibers and of an outer layer consisting of thick bundles of collagenous fibers arranged predominantly in a circular manner. In others this sharp separation of the muscular from the fibrous layer is not evident and only one layer is present, consisting of few muscle cells and abundant bundles of collagenous fibers. In still others an intermediate condition exists: the muscle cells form a distinct inner layer, but some muscle cells are also found in the outer fibrous layer. There is marked individual variation in the number of thin elastic fibers in the media, but there is always a direct relationship between the distribution of the elastic fibers and the smooth muscle cells. When the media consists of two separate layers, the elastic fibers are found almost exclusively in the inner muscle layer. When muscle cells are present throughout the entire

media, elastic fibers are found similarly distributed. The adventitia is from three to five times as thick as the media. It consists of loosely arranged bundles of collagenous fibers, chiefly in a longitudinal fashion, and of thick longitudinally distributed elastic fibers. The external part of the adventitia, which is termed by some "periadventitia," is composed of loose connective tissue with few elastic fibers; it contains the major part of the vasa vasorum.

Results—Autografts

Fresh segments of inferior vena cava were used for transplants to the thoracic aorta in six dogs. Two dogs (Nos. 500 and 556) died 12 and 19 hours after operation. No cause of death was established at autopsy. One dog (No. 552) died in three days of empyema and peritonitis and two dogs (Nos. 657 and 658) died in three and four days respectively of pneumonia. Dogs 556, 657, and 658 died during the night and autopsy was not performed until some hours after death. Dog 545 survived in good health and was sacrificed 190 days after operation.

All of the grafts were patent and had been functioning well. In two (Nos. 552 and 658) a small mural thrombus was present at one suture line; there were no thrombi in the remainder. One graft was 0.6 cm. long; the others varied in length from 1 to 2.1 cm.* The grafts of least age were normal in appearance except for the deposition of some fibrin in the external part of the adventitia of one and a thin layer of fibrin on parts of the intimal surface of the other. The grafts three and four days in age appeared to be undergoing necrosis. Though collagenous and elastic fibers stained normally, the nuclei of the cells exhibited degenerative changes and in general did not stain well. There was some infiltration with blood, fibrin, bacteria, and polymorphonuclear leukocytes.

Before Dog 545 was sacrificed an aortogram was made; it showed some dilatation of the transplant (Fig. 1). Blood pressure was 172/100 in the carotid, 144/100 in the femoral artery. The age of the graft was 190 days, its length 2.1 cm. The internal surface was smooth and no thrombi were present (Fig. 12A). The wall of the graft was as thick as that of the aorta. Microscopically, the intimal surface was covered by endothelium. Graft and aorta were connected at the suture lines by fibrous scar tissue, the collagenous bundles and cells of which were arranged longitudinally. At one suture line the inner surface of this scar tissue was covered by a subendothelial layer of numerous long spindle-shaped connective tissue cells and few thin collagenous fibers, both arranged longitudinally. This subendothelial layer passed over to the vein, covering about one-third of its length while it steadily decreased in thickness from the suture line towards the mid-portion of the graft. Under the endothelium and the subendothelial

* The recorded lengths of all the grafts were from measurements made of the fixed specimen. Considerable shortening occurred after removal of the aorta from the body and still further shortening after fixation and shrinkage.

layer respectively was found the media which was somewhat compressed, chiefly in the fibrous portion. The circularly disposed smooth muscle cells were distinct but not hypertrophied. Elastic fibers could not be seen in the media. The adventitia was extremely compressed; the collagenous tissue was dense and hyalinized and contained few connective tissue cells. The elastic fibers were reduced in number and were fragmented. The next layer was five or six times the thickness of the original graft and was composed of newly formed connective tissue with few cells but abundant collagenous fibers (Fig. 2). All these collagenous bundles were arranged longitudinally in contradistinction to the more interlaced arrangement of normal venous adventitia. Elastic fibers were not seen in the newly formed layer of connective tissue, which was more loosely arranged and stained lighter than the bundles of the original adventitia. Although the individual layers of the graft—endothelium, subendothelial layer, media, adventitia, and newly formed fibrous layer—were easily distinguished, they seemed firmly bound together.

Fresh homografts

In four experiments a fresh segment of the inferior vena cava was transplanted to the thoracic aorta of another dog. One dog (No. 564) died 2½ days later; hemothorax was present without obvious cause for the bleeding. A second animal (No. 705) died of pneumonia during the night of the 38th day; in both these animals autopsy was performed several hours after death. The other two animals (Nos. 565 and 517) survived in good health and were sacrificed in 74 and 148 days respectively.

The grafts varied in length from 0.9 to 1.9 cm. The youngest graft had a smooth intimal surface. It showed degenerative changes in the nuclei of muscle and connective tissue cells though some of the nuclei were stained. There was no visible endothelium, the internal surface being covered by a thin layer of fibrin. The outer surface was covered by a thick layer of blood and fibrin and there was some polymorphonuclear leukocytic infiltration from the outside. The grafts 38 and 74 days in age had a smooth internal surface and no thrombi were present. In the first the vein was composed of collagenous bundles and fragments of thin elastic fibers and was infiltrated by a large number of polymorphonuclear leukocytes and fibroblasts. No muscle cells were present. The inner surface of the mid-portion of the graft was covered by a thin layer of fibrin while a layer of young connective tissue covered the suture lines and extended onto the adjacent one-third of the surface of the vein, steadily decreasing in thickness towards the center of the graft. Newly formed connective tissue, in some places infiltrated with polymorphonuclear leukocytes, covered the outer surface of the graft in a thick layer. There was a moderate relative stenosis at the distal suture line in the case of the 74-day-old graft. The original vein could be seen as a middle layer of the wall, surrounded by newly formed fibrous layers on both

inner and outer surfaces. Of the original vein only collagenous tissue with scanty fragments of thin elastic fibers was preserved; no muscle cells were present. Some connective tissue cells penetrated the original vein wall. The whole inner surface was covered by a layer of young connective tissue varying in thickness from one-half to three times that of the original vein, and this tissue covered the suture lines and was very thick at the distal end where it produced the relative stenosis referred to previously. The connective tissue cells and collagenous fibers were arranged longitudinally in the superficial part and irregularly in the deeper portion, and the entire layer was covered by endothelium. The outer layer was three to five times as thick as the original vein and was composed of few connective tissue cells and many collagenous, partly hyalinized, bundles which were disposed longitudinally in the thinner and were interlaced in the thicker parts.

Before Dog 517 was sacrificed an aortogram was made; it revealed good patency of the grafted area (Fig. 3). The femoral blood pressure was 160/120, the carotid pressure 212/130. The age of the graft was 148 days, its length 1.9 cm. The internal surface was smooth except at one suture line where a piece of silk had protruded into the lumen and become surrounded by a small thrombus. The wall of the graft was considerably thickened. Microscopically, the original vein was best seen near the suture lines where it was composed of a continuous layer of collagenous bundles and some fragments of elastic fibers (Fig. 4). No muscle cells were present. The collagenous bundles were somewhat swollen and could be easily distinguished from those in the adjacent newly formed connective tissue. Some fibroblasts were found among the bundles. The mid-portion of the original vein consisted only of short pieces of the original collagenous bundles which were surrounded by abundant fibroblasts and newly formed collagenous fibers. Between these structures there was some amorphous material which took a pale grey-blue stain with hematoxylin and eosin and which probably represented partly disintegrated collagen; in addition there were some fibrous structures, probably representing elastic fibers, which were impregnated with calcium and separated by clefts. The original vein was surrounded on the outside by a wall of cellular granulation tissue, the outer portion of which was rich in connective tissue and showed perivascular lymphocytic infiltration at the border of the normal surrounding tissue. On the inner surface of the original vein there were several cushions of degenerated tissue partly fibrous and calcified, but composed mainly of an amorphous material without nuclei, which took a grey-blue stain with hematoxylin and eosin. This mass was covered by a ribbon of hyaline material containing some connective tissue cells and probably representing old fibrin and, in addition, a layer of young connective tissue arranged longitudinally. The same tissue covered the suture lines. The whole layer was covered by endothelium. There was a small thrombus at one point on the surface.

Homografts preserved by freezing

In six experiments a segment of inferior vena cava was preserved for from 3 to 33 days in the frozen state before being transplanted to the thoracic aorta of another dog. One dog (No. 621) died at the close of the operation, another (No. 603) after 36 hours from empyema, a third (No. 646) after 20 days from empyema and pneumonia. Three (Nos. 614, 637, and 639) survived in good health and were sacrificed after 7, 55, and 77 days respectively.

The graft in the dog which died at the close of the operation had been frozen for 16 days. Microscopically it looked normal except for some pyknosis of the nuclei of muscle and connective tissue cells, apparent loosening of the collagenous bundles, and a thin layer of fibrin on the external surface. The transplant, 36 hours in age, was occluded by a thrombus and microscopically appeared to be undergoing destruction with no visible nuclear staining and no recognizable muscle cells. The 7- and 20-day-old grafts showed some thickening of the wall. In the younger there was interposed between the original graft and the tissue of the host a layer of fibrin with polymorphonuclear leukocytes and fibroblasts, while in the older there was a much thicker layer of newly formed fibrous tissue, fibrin, leukocytes, and detritus. In both there was evidence of destruction of the transplant with no staining of nuclei, no recognizable muscle cells, fragmentation of collagenous bundles and elastic fibers; in both the adventitia appeared loosened by edema fluid.

Before Dog 637 was sacrificed aortograms were made; they revealed good patency of the graft (Fig. 5). Blood pressure was 220/138 in the femoral, 206/140 in the carotid artery. The graft had been frozen 33 days; its age was 55 days, its length 2.0 cm. The internal surface was partly smooth, partly rough (Fig. 12B); no thrombi were present. The wall was thickened. Microscopically, the graft appeared to be undergoing destruction (Fig. 6). No nuclei were stained, no muscle cells were present, and the remnants of media and adventitia could not be distinguished separately. In the inner part of the original graft the collagenous bundles were finely fragmented and were separated by edema fluid and dense fibroblasts. Some of the fibroblasts lying in the longitudinal direction of the graft near elastic fibers were very long and spindle-shaped. There was a dense network of delicate newly formed collagenous fibers. The elastic fibers appeared pulled apart, fragmented, and reduced in number. In the outer portion of the original graft only small remnants of collagenous bundles and tiny fragments of elastic fibers were preserved; they were surrounded by young connective tissue with abundant collagenous fibers. Near the suture line, infiltration of the graft with fibroblasts had not yet occurred, and the collagenous bundles and elastic fibers were better preserved. The inner portion of the suture lines was filled with young connective tissue and this tissue

covered the adjacent part of the graft and was growing into the layer of fibrin which lined the middle of the graft.

Before Dog 639 was sacrificed an aortogram was also made and normal patency was demonstrated. This graft had been frozen 33 days, was 77 days in age and 2.5 cm. in length. Blood pressure was 216/144 in the femoral and 204/144 in the carotid artery. The internal surface was smooth except for a small thrombus at one point. The wall was thickened. As in the others, the graft appeared microscopically to be undergoing destruction with no staining of nuclei and no recognizable muscle cells. The collagenous bundles of the media were normally close together. The inner part of the adventitia was closely packed. The collagenous bundles were broken up into small pieces and the elastic fibers were fragmented but not pulled apart. Some fibrin was present in this part of the adventitia. The outer part of the adventitia was markedly loosened; thin collagenous bundles and remnants of elastic fibers were present. The original graft was only moderately infiltrated with fibroblasts, and there was but a slight connective tissue formation on the external surface of the graft. In the newly formed connective tissue there was a tiny abscess, composed of polymorphonuclear leukocytes and round cells. The inner portion of the suture lines was filled with connective tissue which covered the adjacent part of the graft and was growing into a layer of fibrin which lined the middle of the graft.

Formalin-fixed homo- and heterografts

In eleven animals venous grafts which had been fixed in formalin for weeks or months were transplanted to the thoracic aorta. In seven animals homografts of inferior vena cava were used, in two (Nos. 372 and 435) homografts of innominate vein, and in two (Nos. 598 and 547) heterografts of human common iliac vein secured at autopsy. One dog (No. 536) died of unknown cause about 4 hours after completion of operation; four others (Nos. 615, 619, 496, and 544) died after 22, 24, 48 hours, and 20 days respectively without demonstrable cause being evident at autopsy, while a sixth (No. 372) died of pneumonia after 22 days. Dogs Nos. 516, 421, 598, 547, and 435 survived in good health, except for paraplegia in No. 421, and were sacrificed after 5, 88, 121, 133, and 305 days respectively. The grafts varied in length from 1.5 to 4.0 cm.

The four grafts ranging in age from a few hours to two days showed the same features. The intimal surface was smooth. The tissues underneath the lining membrane had a reddish-blue color due to hemorrhage in the adventitial layer. Microscopically, the nuclei, muscle cells, and collagenous and elastic fibers were stained normally. There was some deposition of fibrin on the suture line and to varying degrees on the intimal surface of the graft where it filled small defects extending into the media-like plugs. There was some polymorphonuclear infiltration. In the 2-day-old graft the nuclear stain was pale as compared with that of the adjacent aorta and the

infiltrating leukocytes. This was also a feature of the graft 5 days old. The wall of the 20-day-old transplant was definitely thickened. Microscopically it was quite like the 22-day-old graft from Dog 372.

This graft was 22 days old. It was 2.3 cm. long. The internal surface was smooth and the wall of the transplant was thickened (Fig. 12C). The injected specimen showed the graft and the aorta to be roughly equal in size (Fig. 7). There was some fibrin on the suture line. Microscopically, nuclear stain and muscle cells were absent. Collagenous and elastic fibers were stained normally. At points on the internal surface there was some fibrin which was penetrating into the spaces between the underlying collagenous bundles of the media. The inner portion of one suture line was filled with young connective tissue which also covered the adjacent graft for a short distance. The outer portion of both suture lines was filled with connective tissue. The media showed compact collagenous bundles. The inner layer of the adventitia was separated from the outer layer, pulled apart, and infiltrated by a young cellular connective tissue. The outer part of the adventitia showed only remnants of collagenous bundles surrounded by young connective tissue rich in long, spindle-shaped fibroblasts and delicate collagenous fibers. The elastic fibers were well preserved in the greater portion of the adventitia, whereas they were fragmented and reduced in number and thickness in a smaller part. There was old fibrin at several points in the adventitia and on its external surface. The adventitia was surrounded by a thick layer of newly formed connective tissue.

The 88-day-old graft also showed a smooth internal surface, considerable thickening of its wall, normal staining, and recognizable muscle cells. The inner surface was partly covered by a thin layer of fibrin and there was some connective tissue extending from the suture lines onto the graft and growing into the layer of fibrin. Between this layer and the original graft were some cavities filled with blood, fibrin, and a homogeneous material. The collagenous bundles of the original adventitia were fragmented but preserved. The elastic fibers were fragmented and rather thick but not apparently reduced in number. The adventitia was surrounded by a thick layer of newly formed firm connective tissue. The 121- and 133-day-old grafts showed essentially the same features except that the accumulation of blood and fibrin between layers was not so conspicuous and only scanty remnants of elastic fibers were present. Before sacrificing the animals with these grafts (Dogs 598 and 547) blood pressures were obtained. In the first the carotid pressure was 157/107 and femoral pressure 160/96; in the latter these values were 131/118 and 154/112. In one (Dog 598) there was a spindle-shaped dilatation of the transplant but it should be noted that at the time of its insertion this transplant was considerably greater in diameter than the aorta. In the other dog an aortogram was made (Fig. 8).

Before Dog 435 was sacrificed an aortogram was also made; it revealed a patent graft (Fig. 9). Femoral blood pressure was 150/100, carotid

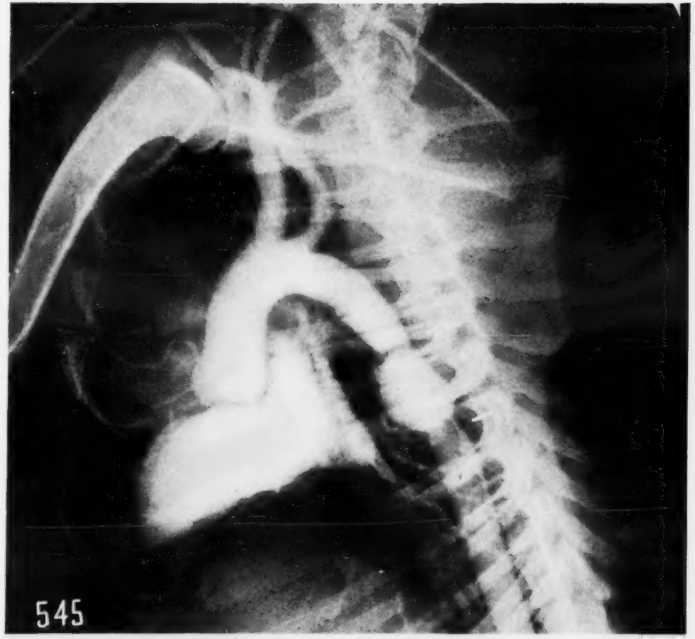

FIG. 1. Aortogram in Dog 545 (autograft) made 190 days after transplantation. Note slight dilatation of graft. At time of original operation the transplant was slightly smaller in diameter than the aorta.

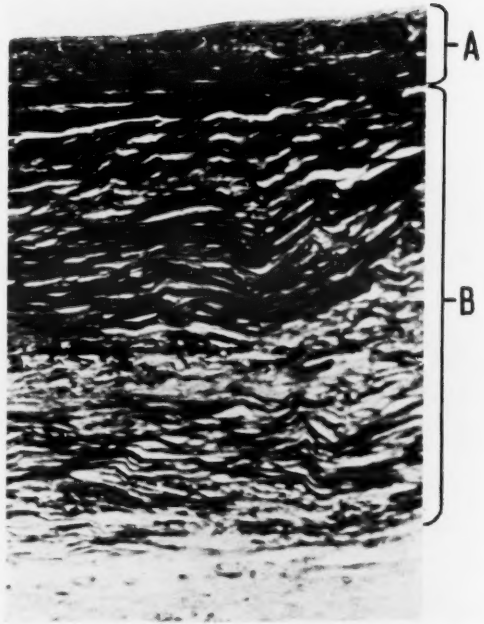

FIG. 2. Photomicrograph of specimen from Dog 545, a fresh autograft 190 days old. Paraffin. Verhoeff-Van Gieson stain. Longitudinal section of mid-portion of graft. The original graft is well preserved but somewhat compressed. There is a thick layer of newly formed fibrous tissue. A. Original graft. B. Newly formed fibrous layer. Enlarged 280 times.

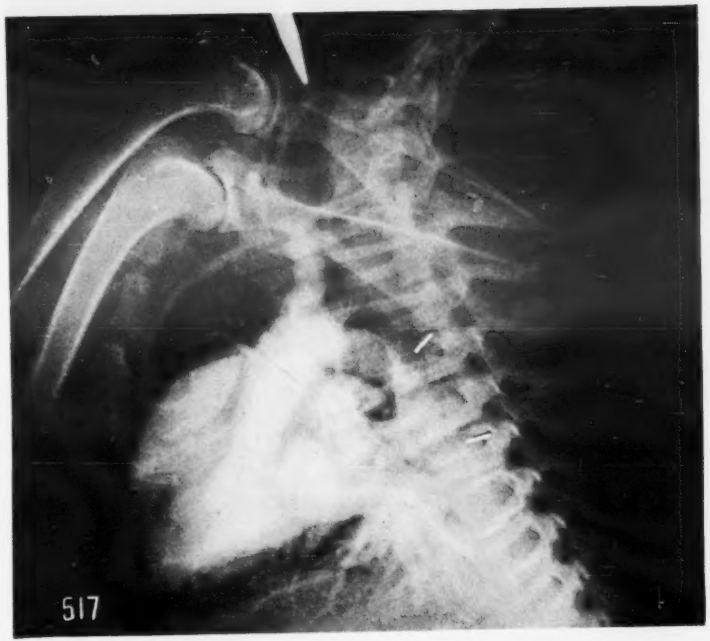

FIG. 3. Aortogram in Dog 517 (fresh homograft) made 148 days after transplantation. There is no dilatation of the transplant, the limits of which are marked with white lines.

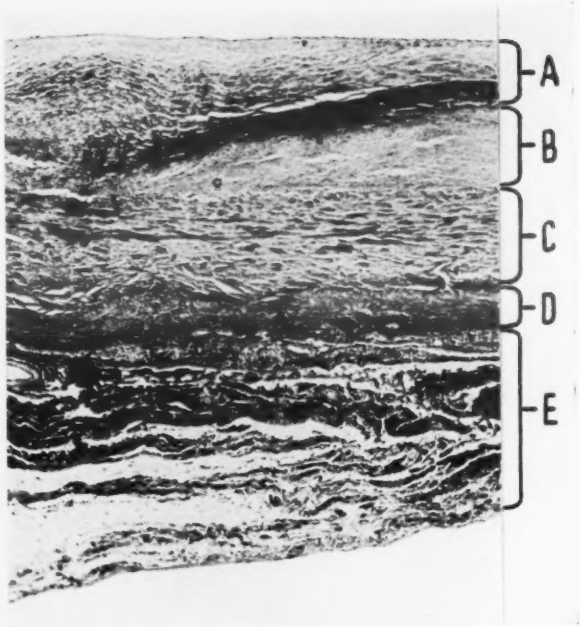

FIG. 4. Photomicrograph of specimen from Dog 517, a fresh homograft 148 days old. Paraffin. Verhoeff-Van Gieson stain. Longitudinal section of mid-portion of graft. The original vein consists of fragments of collagenous bundles (dark) surrounded by fibroblasts and newly formed collagenous fibers. On the internal surface there is a cushion of degenerated tissue, a ribbon of hyaline material (dark), and a layer of young connective tissue. On the external surface there is a wall of granulation tissue and fibrous tissue. A. Young connective tissue and ribbon of hyaline material (dark). B. Cushion of degenerated tissue. C. Original vein. D. Granulation tissue and fibrous tissue. E. Adherent tissue of the host. Enlarged 43 times.

FIG. 5. Aortogram in Dog 637 (preserved homograft) made 55 days after transplantation. The diameter of the graft appears slightly greater than that of the aorta. At the time of the original operation the transplant bulged out to a diameter roughly 50 per cent greater than that of the aorta.

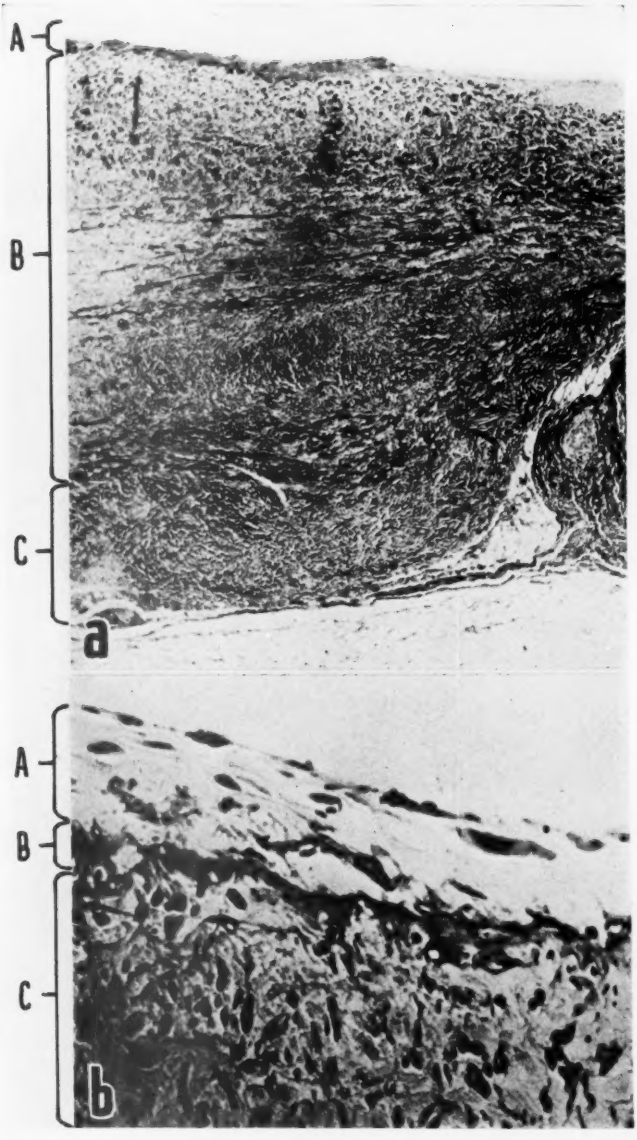

FIG. 6. Photomicrographs of specimen from Dog 637, a frozen homograft 55 days old. Paraffin.

a. Verhoeff-Van Gieson stain. Longitudinal section of mid-portion of graft. The internal surface is covered on the left by fibrin, on the right half by young connective tissue. The graft is densely infiltrated with fibroblasts. On the external surface is a layer of young connective tissue incorporating some of the collagenous bundles of the original graft. A. Fibrin and young connective tissue. B. Original graft with new formation of connective tissue. C. Newly formed fibrous layer. Enlarged 38 times.

b. Verhoeff-Masson stain. Higher magnification. Fibroblasts are seen growing over the layer of fibrin which covers the internal surface of the graft. A. Young connective tissue. B. Fibrin. C. Original graft with many fibroblasts. Enlarged 555 times.

FIG. 7. Roentgenogram after injection of diodrast into left ventricle of specimen removed from Dog 372 (formalin-fixed homograft) 22 days after transplantation. The graft is slightly larger in diameter than the aorta, as was the case at the time of its insertion (outside diameter of graft 1.5 cm.; aorta 1.2 cm.)

FIG. 8. Aortogram in Dog 547 (formalin-fixed heterograft) made 133 days after transplantation. The graft and aorta are about equal in diameter; at the time of insertion the transplant was somewhat greater in diameter.

FIG. 9. Aortogram in Dog 435 (formalin-fixed homograft) made 305 days after transplantation. The comparative diameters of graft and aorta are about the same as was the case at the time of insertion of the transplant.

FIG. 10. Roentgenogram of specimen removed from Dog 435 (formalin-fixed homograft 305 days in age). Note the enormous thickening of the wall of the transplant. Some areas of calcification are seen.

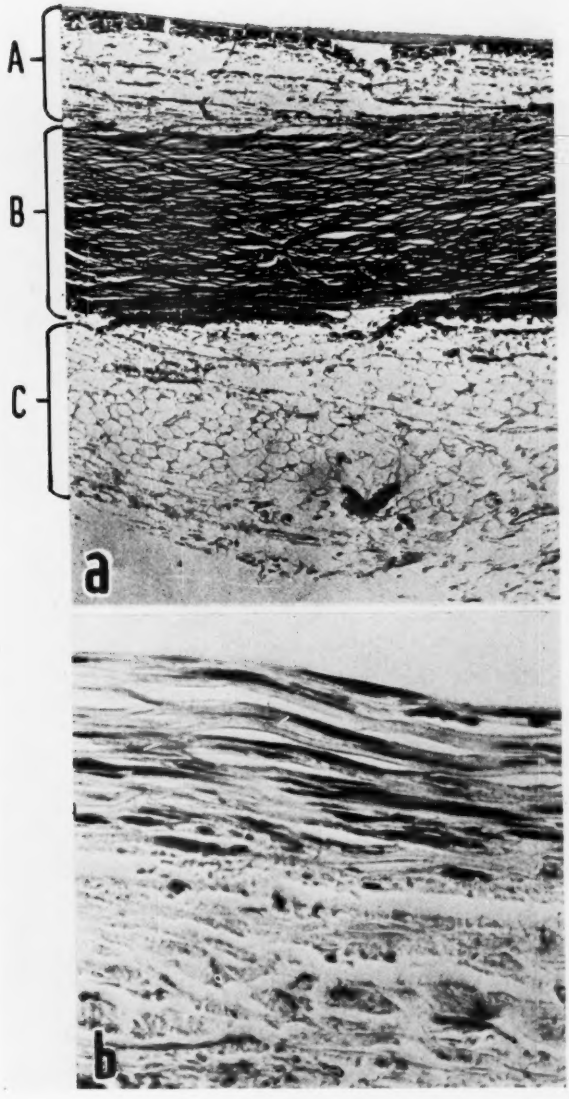

FIG. 11. Photomicrographs of specimen from Dog 435, a formalin-fixed homograft 305 days old. Paraffin.

a. Verhoeff-Van Gieson stain. Longitudinal section of mid-portion of graft surrounded by newly formed external fibrous layer. A. Original graft. B. Newly formed fibrous layer. C. Adherent tissue of the host. Enlarged 55 times.

b. Masson stain. Higher magnification. Newly formed layer of connective tissue on internal surface of graft. Elongated fibroblasts are seen. Enlarged 800 times.

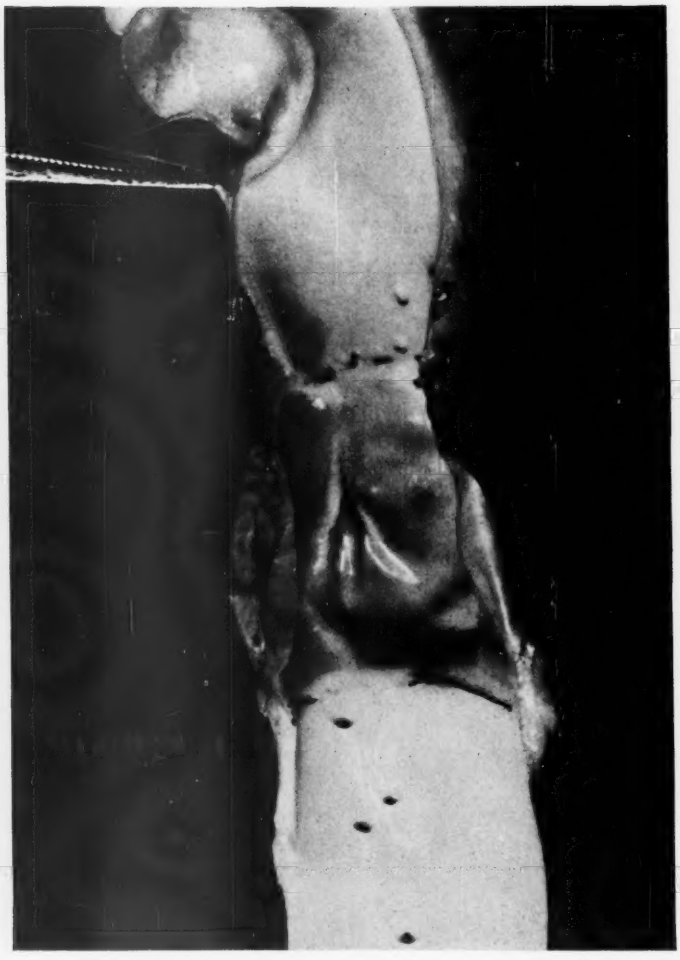

FIG. 12A. Venous transplant to the aorta. Photograph of specimen from Dog 545, a fresh autograph 190 days in age. Note shiny inner surface of graft and thickened wall of graft.

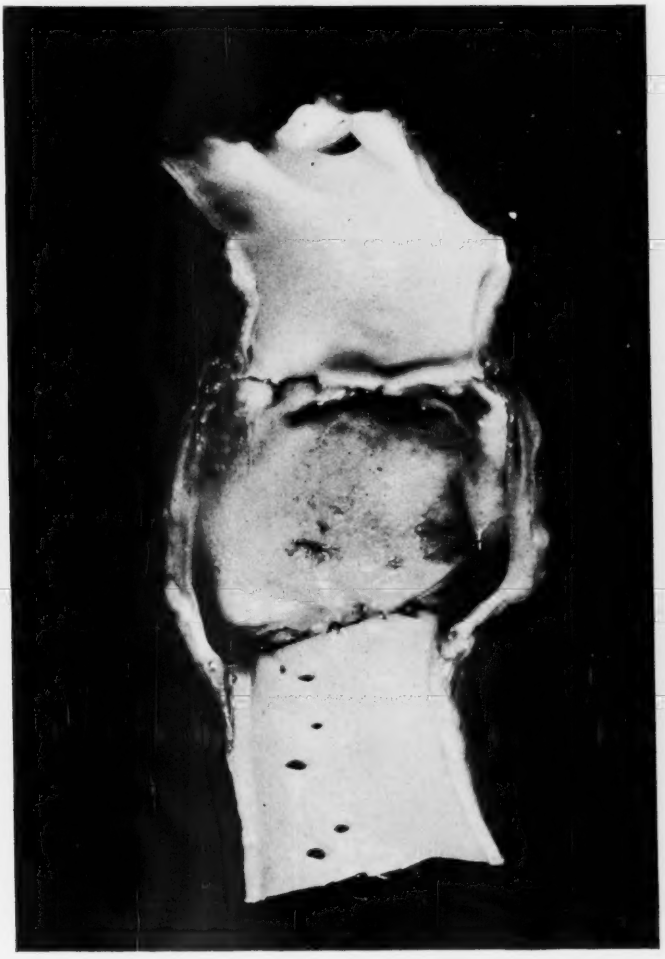

FIG. 12B. Venous transplant to the aorta. Photograph of specimen from Dog 637, a preserved homograft 55 days in age. The internal surface of the graft is seen to be generally smooth but to have a few roughened granular areas. Note thickening of wall of graft. The difference in circumference of the graft and aorta is somewhat less than was noted at time of the transplantation.

FIG. 12c. Venous transplant to the aorta. Photograph of specimen from Dog 372, a formalin-fixed innominate vein homograft 22 days in age. Note the thickening of the wall of the transplant.

pressure 142/102. The age of graft was 305 days, its length 4.0 cm. The internal surface was partly smooth, partly covered by a thin layer of fibrin. At one point there were some yellow spots under the internal surface. The wall of the graft was thickened (Fig. 10). Microscopically, nuclear stain and muscle cells were missing (Fig. 11), while collagenous and elastic fibers were stained normally. The middle portion of the internal surface of the graft was covered by a thin layer of fibrin. The suture lines were filled with young connective tissue which also extended for some distance onto the surface of the graft and was growing into the fibrin. A continuation of the endothelium of the connective tissue layer covered the adjacent part of the fibrinous layer but not the middle portion of the fibrinous layer. There was some fibrin in the spaces between the collagenous bundles of the original media. The original adventitia was a loose layer composed of remnants of collagenous bundles and elastic fibers. This layer was surrounded by newly formed firm connective tissue, the collagenous bundles of which were arranged in the longitudinal direction of the graft. There was no newly formed connective tissue in the original media and adventitia. Only a few lymphocytes were seen infiltrating the original graft.

Discussion

These studies demonstrate that fresh venous autografts and homografts, venous homografts preserved by freezing, and homo- and heterografts previously fixed in formalin are all capable of functioning effectively to bridge defects in the thoracic aorta. In the majority of instances the specimens, when examined from a few hours to ten months after transplantation, showed a smooth lining membrane and considerable thickening of the wall of the graft. In a few instances a small mural thrombus was present, but only in one of the 27 transplants was there a thrombus which occluded the lumen. This exceptional case was a dog which died of massive empyema 36 hours after transplantation of a homograft preserved by freezing. In only one case was there a minute aneurysmal dilatation adjacent to the suture line. In several instances small portions of the silk suture used for the anastomosis were visible inside the lumen of the graft. Frequently there was no thrombus over the protruding suture material but in some instances a mural thrombus appeared to have originated in such a manner. Since the completion of the experiments reported in this communication, experiments have been carried out in which interrupted silk sutures were used instead of a continuous suture; in these experiments such protrusion of suture material into the lumen has not occurred, and it is believed that the use of interrupted sutures will diminish considerably the hazard of stenosis and probably also of mural thrombi.

At the time of the post-mortem examination most of the grafts had comparatively the same diameter with reference to the aorta as was noted at the completion of the operative transplantation. In some instances where

the venous insert was of larger diameter than the aorta, it appeared that the diameter of the graft had decreased slightly so that the size of the aorta and graft was more nearly the same. In one instance a moderate dilatation of the graft had apparently taken place.

In the present study we have expanded histological observations of certain types of grafts, only single observations of which have been previously reported. In a survey of the literature,[16] histological descriptions of only one fresh venous homograft and one formalin-fixed venous homograft were found, and none of a formalin-fixed venous heterograft.

The only venous grafts which had the appearance of living structure were the fresh autografts. Only one of these was of relatively great age at the time of examination (190 days). Even this specimen showed signs of retrogressive change apparently due to the stress of the arterial pressure: the elastic fibers were reduced in number and were fragmented, the fibrous portion of media and adventitia was markedly compressed, and the fibrous part of the adventitia was undergoing hyaline change. The muscle cells of the media, however, looked unchanged. They were not hypertrophied.

This autograft showed the same two changes which were found in all other types of grafts: formation of an external and internal fibrous layer. The former is the most important feature in all types of venous grafts, since it gives strength to the transplant in spite of such retrogressive changes as occur in venous autografts and in spite of degeneration of the entire original vein in all other types of venous grafts. The formation of this fibrous reinforcement on the outside is very important in the functional success of vascular transplants.

The origin and nature of the newly formed fibrous layer on the internal surface of the graft have been the subject of much debate in the past.[10] Some earlier investigators have felt that this layer is derived from the intima of the graft. However, this origin could be expected only in autografts. The opinions were divided as to whether this layer was of compensatory origin due to the arterial pressure or of inflammatory origin due to lesions of the internal surface of the graft made during operation. The impression obtained from study of the grafts described in this present communication may be summarized as follows:

In all types of grafts, including autografts, this layer grows tongue-like from the suture lines towards the middle of the graft and is formed by the young connective tissue which fills the inner funnel-like space at the suture lines. In the case of the 190-day-old autograft this was nicely proved by the fact that only one suture line was filled with connective tissue and only on this side was there a thin layer of connective tissue growing over the graft. If the graft is short, the two "tongues" of connective tissue join each other in the middle so that the whole internal surface is covered by a fibrous layer. If the graft is longer, only the ends are covered even after a considerable length of time, whereas the mid-portion of the graft is, in most

cases, covered by a thin layer of fibrin. It seems that this fibrin promotes the formation of a fibrous layer by furnishing a sort of foothold for the ingrowing fibroblasts, a condition well known from other pathological processes. In some cases there was evidence that the fibroblasts were actually growing into the fibrin (Fig. 6b) or that remnants of fibrin were preserved in the fibrous layer. A similar process takes place in the healing of all blood vessels at the suture line: the funnel-shaped space at the suture lines is first filled with a fibrin plug which later becomes organized. It seems natural that the formation of such an internal fibrous layer may reinforce the graft. However, since this process does not develop as early or regularly as the external fibrous layer, it is apparently less important in this respect.

It has been stated by some earlier investigators[16] that the fibroblasts of the internal fibrous layer are transformed into smooth muscle cells. Such a fact would be of great theoretical importance, since the postembryonic transformation of fibroblasts into smooth muscle cells has not been established.[7] It is generally agreed that the smooth muscle cells of newly formed blood vessels associated with healing processes, e.g. pleural adhesions, are derived from the perivascular embryonic cells of the adult, but these cells are more primitive than fibroblasts. There was no evidence in the sections of the grafts studied that such a transformation took place. The fibroblasts of the internal fibrous layer sometimes showed a very elongated spindle-shaped form which might have induced earlier investigators to consider them as smooth muscle cells. However, the assumption of such an elongated form under pressure, in this case arterial pressure, is a well known fact. Fibroblasts which were seen penetrating into the original graft also assumed this elongated form but again without signs of transformation into smooth muscle cells as had earlier been thought to be the case.[12, 13] There was no evidence to suggest the formation of elastic fibers in the internal fibrous layer, another occurrence sometimes described by earlier workers.[16]

The difference in the histological fate of fresh, preserved and formalin-fixed homografts and of formalin-fixed heterografts did not appear to be very remarkable. The disintegration of the original vein seemed to be perhaps greater in homografts preserved by freezing than in fresh homografts. The end result of formalin-fixed grafts, too, appeared histologically to be inferior to that of fresh homografts. The internal surface of formalin-fixed grafts seemed particularly breakable and permitted the penetration of blood and fibrin into the wall. In several instances fibrin was seen to fill small defects of the surface like plugs and to extend between the collagenous bundles to the media. In some cases, fibrin, blood, and edema fluid were found in the original graft, and in two cases calcification was seen grossly. The impression was held that in the two formalin-fixed heterografts the disappearance of the elastic fibers was more complete than in formalin-fixed homografts of the same age. The results in formalin-fixed

grafts described in this paper are essentially the same as those recently reported by Peirce and his associates[15] who used formalin-fixed thoracic aortas of dogs for transplantation into abdominal aortas or for extracardiac shunts in dogs. They found in specimens of ten dogs, ranging in age from two days to nine months, degeneration of the original graft and formation of an internal and external fibrous layer. There was calcification within the graft wall in four specimens.

The histological fate of blood vessel grafts previously fixed in alcohol or formalin has been a subject of lively discussion in the past. Nageotte stated that he observed in grafts of fixed blood vessels[12, 13] as well as in grafts of fixed tendon[6–11] that the connective tissue cells were dissolved and replaced by fibroblasts of the host, whereas the collagenous fibers, which Nageotte considered as always being dead material similar to crystals, were preserved and had the same relationship to the new fibroblasts as to the original ones. Nageotte called this process "reviviscence" of the connective tissue. This theory has been repudiated with reference to grafts of fixed tendon by Busacca[1, 2, 3] and Weidenreich.[18] It could be seen in the formalin-fixed grafts described in this paper that the collagenous bundles were slowly dissolved and replaced by collagenous fibers formed by the immigrated fibroblasts of the host.

Our studies confirm the general opinion reached by the majority of earlier workers that, although various types of vascular transplants function well, only fresh autogenous grafts survive as a living structure. Little difference was noted in the gross appearance of the various types of venous transplants, fresh autografts and homografts, preserved homografts, and formalin-fixed homo- and heterografts. Microscopically, however, all except the first type appeared to undergo destruction. It should be pointed out that our observations were limited to specimens ten months or less in age. Although there are in the older literature a few examples of specimens studied years after transplantation, it would seem most important to expand our knowledge of the long-term behavior of vascular transplants. It is perfectly possible that the incidence of such complications as aneurysmal dilatation or rupture might be more numerous after the passage of a long period of time. It would also seem essential to study the fate of vascular transplants in the growing animal in order to determine whether the graft increases in diameter as the parent vessel grows in size, or whether it tends to remain relatively fixed in diameter with the eventual production of a condition of relative stenosis. Some of these problems are currently being investigated.

The use of free transplants of fresh autogenous veins has already proved itself to be a satisfactory means of bridging gaps in peripheral arteries in man.[17] Gross and his associates have recently reported good functional results with the use of arterial homografts preserved at a cool temperature in a specially buffered nutritive medium in bridging aortic defects in man

and in creating systemic-pulmonary shunts in cases of tetralogy of Fallot.[4, 5, 9, 14] Full reports of their experimental observations with such grafts and particularly of their histological studies will be awaited with great interest. It may be expected, however, that these preserved homografts behave like fresh homografts when they are implanted into the host.

Our experiments appear to offer no conclusive data for application in clinical practice except to point out that only fresh autografts are capable not only of good function but of survival as living transplants. The other types of grafts apparently yield satisfactory functional results at least for reasonably short periods of time. Until more adequate data are available concerning long-term results, it would seem wise to use fresh autografts whenever possible.

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A RAPID METHOD FOR DEMONSTRATING THE ACTION OF STAPHYLOCOCCUS ENTEROTOXIN UPON *RANA PIPIENS**

ELIZABETH D. ROBINTON†

Studies of staphylococcal food poisoning have been made difficult by the lack of an easy, reliable method for demonstrating the presence of an enterotoxin, elaborated by certain strains of *Staphylococcus aureus* (*Micrococcus pyogenes*). The commonly used laboratory animals (guinea pig, mouse, rabbit, rat) are rodents, which as a class lack the appropriate mechanism for vomiting. These animals, therefore, are valueless in this study since the presence of the enterotoxin in any substance is demonstrated by its ability to elicit a vomit reaction. At present, the usual method for the demonstration of the enterotoxin is to administer either the suspected foodstuff or a filtrate prepared from a strain of *Staphylococcus aureus* isolated from suspected food, to human volunteers, monkeys, kittens, or cats, all of which are known to possess the vomit mechanism. Slocum[5] has clearly summarized the limitations of these three tests, reporting that the use of human volunteers is not without danger, that monkeys are expensive and difficult to house, and that kittens and cats are most difficult to obtain and maintain in confinement. He further points out that each of these species not only shows variation in susceptibility to the enterotoxin, but also is capable of developing resistance to it upon repeated administration.

The common frog, *Rana pipiens*, an inexpensive and readily available animal which is known to possess a vomit mechanism, was chosen for further study by Robinton.[4] It was shown that upon administration of a known staphylococcal enterotoxin, a physiological reaction was obtained, designated as a spasm, which was an outward manifestation of an antiperistaltic action of the stomach. This method, however, has proven of limited application because of the apparently uncontrollable time lapse between the administration of the enterotoxin and the occurrence of the spasm which indicates a positive reaction.

Mellinger[3] reported that the frog was capable of vomiting by the action of the stomach alone without the aid of other structures. Upon administration

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* From the Department of Public Health, Yale University, and the Department of Hygiene and Bacteriology, Smith College. The data presented here are taken, in part, from a dissertation submitted to the Graduate School of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Aided by a grant from the James Hudson Brown Memorial Fund of the Yale University School of Medicine.

† Present address: Department of Hygiene and Bacteriology, Smith College, Northampton, Massachusetts.

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of tartar emetic to the decerebrate frog, he was able to show increasing antiperistalsis from the pylorus toward the cardia with expulsion of the stomach contents through the mouth. Using apomorphine, similar results could be obtained, although vomiting never occurred because paralysis of the musculature due to the action of the drug set in to prevent it. No substances other than emetics gave a similar picture. Mellinger also noted seasonal variation among the reacting animals, reporting that frogs used in June and July were best, that the vomiting ability decreased in October and November and was absent in January and February.

This phenomenon of seasonal variation was noted in the present study when it was observed that the spasms were occurring with less frequency following administration of an enterotoxin during the month of November and were completely absent in December and January.

Procedures

The present investigation was carried out in an attempt to repeat Mellinger's work and to extend it to include the use of staphylococcal enterotoxin. In an effort to overcome seasonal variation in the frogs, all animals received during the winter months were maintained in a laboratory at a temperature of 80-85° F. and fed every 48 hours with fresh ground lean beef. It is essential that the animal be healthy and in a good nutritive state, for it has been accepted since Cannon's report in 1912[1] that only when the stomach muscle is in a condition to be stretched (tonus) can rhythmic contraction be obtained.

Decerebration was performed by cutting through the upper jaw at an angle directly behind the eyes and in front of the tympanic membranes; the medulla and spinal cord were thus left intact. The blood which had collected at the mouth was removed with a small cotton pledget. The animal was then placed on its back and fastened securely with pins upon a cork board. The abdominal wall was opened and the stomach exposed with both pyloric and cardial ends visible. Each frog had been fed ground lean beef four hours prior to testing. The meat had undergone a partial digestion at the end of this time, yet the stomach was still full and its contents easily visible due to the discoloration of the meat.

Results and discussion

Two known enterotoxigenic filtrates were used in this study, one of which was prepared according to Dolman's method[2] from *Staphylococcus aureus,* strain ♯196,* and the other being a prepared toxin ♯12069.† Control materials were filtrates prepared from Dolman's semi-solid agar, and from

* Obtained through the courtesy of Dr. Gail Dack, Department of Bacteriology, University of Chicago.

† Obtained through the courtesy of Dr. C. E. Dolman, University of British Columbia, directly from his laboratory.

Staphylococcus aureus, F.D.A. strain ♯209 and Wood strain ♯46.‡ The material to be fed was placed directly into the stomach through the esophagus using a medicine dropper.

The results obtained with both test and control materials are summarized in Table 1.§ When 1% copper sulfate was administered to the frog, an immediate and violent reaction occurred. Antiperistalsis was marked and the stomach contents moved rapidly through the esophagus and into the mouth cavity. When 0.5% tartar emetic was given, the reaction was much

TABLE 1

EFFECT OF VARIOUS SUBSTANCES UPON PERISTALTIC ACTION OF FROG'S STOMACH

Material	*Reaction*	*Time*
Controls		
Culture medium	normal peristalsis	1 hour
Non-enterotoxigenic filtrate ♯209	normal peristalsis	1 hour
Non-enterotoxigenic filtrate ♯46	normal peristalsis	1 hour
Tests		
1% copper sulfate	antiperistalsis	immediate
0.5% tartar emetic	antiperistalsis	5 minutes
Enterotoxigenic filtrate ♯196 (3 frogs)	antiperistalsis	20, 22, 28 minutes
Enterotoxigenic filtrate ♯12069	antiperistalsis	20 minutes

less marked and only antiperistalsis was seen, the contents moving slowly, but definitely, from the pylorus toward the cardia. A sharp ring of constriction was noted at the pylorus while the upper part of the stomach showed complete relaxation. Several sharp peristaltic contractions occurred which began about the middle of the stomach and which moved downward, but at no time did the pylorus open or relax. The stomach at times looked as though it was divided into two portions by such constriction bands. The antiperistaltic wave took place from the constriction ring toward the esophagus and the stomach contents could be seen to move in an upward

‡ Obtained through the courtesy of Dr. Glen Slocum, Food and Drug Administration, Washington, D. C.

§ The author is indebted to Professor Myra M. Sampson, Department of Zoology, Smith College, for her guidance in the preparation of the test animal and for checking the physiological reactions observed.

direction, but only liquid material reached the mouth cavity. It would appear that the tartar emetic had a toxic effect upon the musculature which prevented the complete expulsion of the stomach contents. Culture medium when fed, as well as non-enterotoxigenic filtrates #209 and #46, caused no antiperistaltic waves to occur at any time during an observation period of 60 minutes. The pylorus, in each frog studied, reacted normally as it did when ground beef was fed. However, when enterotoxigenic filtrates #196 and #12069 were used, antiperistaltic movements were clearly seen within 20 to 30 minutes after administration. These motions were definitely toward the cardia and often carried the food into the esophagus. In some animals the constriction of the pylorus was accompanied by a similar constriction of the esophageal sphincter; the esophagus became elongated and completely

TABLE 2

EFFECT OF CONCENTRATED SUBSTANCES UPON PERISTALTIC ACTION OF FROG'S STOMACH

Materials	*Reaction*	*Time*
Controls		
Culture medium	normal peristalsis	1 hour
Non-enterotoxigenic filtrate #209	normal peristalsis	1 hour
Non-enterotoxigenic filtrate #46	normal peristalsis	1 hour
Enterotoxigenic filtrate #196	antiperistalsis	immediate
Enterotoxigenic filtrate #12069	antiperistalsis	immediate

motion was thus mechanically blocked. However, waves of antiperistalsis continued to take place. It appears evident that the enterotoxin is capable of acting in the same way as the tartar emetic in paralyzing the musculature, at least for a period of time.

An attempt was made to obtain a more concentrated enterotoxin by freezing and drying under vacuum (Cryochem process).* Ten ml. amounts constricted, and although movement of food toward it was seen, its forward of semi-solid agar, non-enterotoxigenic filtrates #209 and #46, and enterotoxigenic filtrates #196 and #12069 were concentrated to dryness. When the tests were to be made, the materials were brought back to a volume of two ml. with sterile distilled water. Thus, in each instance the material was

* The author is indebted to Dr. F. L. Mickle, Director, and Dr. Kingston Wilcox, Research Microbiologist, of the Bureau of Laboratories, Connecticut State Department of Health, for their assistance in this process.

concentrated to five times its original potency. Upon administering these to decerebrate frogs, no reactions were obtained within a period of an hour with the culture medium and non-enterotoxigenic filtrates #209 and #46. However, reactions were obtained within one minute after administration of both the #196 and #12069 concentrated enterotoxigenic filtrates. Definite antiperistalsis from the cardia into the esophagus took place rapidly. These results are summarized in Table 2. Thus, it would appear that when enterotoxin is sufficiently concentrated, its action is similar to that of a direct emetic such as copper sulfate.

Conclusions

A rapid test for the demonstration of staphylococcal enterotoxin has been described using the common frog, *Rana pipiens*. It has been shown that antiperistalsis of the stomach in decerebrate frogs occurs only when known emetic or enterotoxigenic substances have been administered, that this reaction occurred in every frog fed with such substances, and that such reactions may be obtained within thirty minutes after feeding. This period of time may be shortened to one minute with the use of concentrated enterotoxin.

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THE EFFECT OF ADRENALECTOMY AND OF THE ADRENOCORTICOTROPHIC AND GROWTH HORMONES ON THE SYNTHESIS OF FATTY ACIDS*

ISAAC D. WELT† AND ALFRED E. WILHELMI‡

The recent work of Stetten and his colleagues[5, 23–26] has drawn attention to the facts that the process of fat synthesis from carbohydrate may often be a major pathway of carbohydrate metabolism in the normal animal, that this process is greatly accelerated by insulin, and that in the diabetic animal the conversion of sugar to fat is almost completely suppressed. This paper is a report of experiments upon certain other endocrine effects on the process of fat synthesis. It is found that in plateaued female albino rats, on a high-carbohydrate, fat-free diet, adrenalectomy is followed by a greatly increased uptake of deuterium in the liver and body fat in an 8-day period during which a constant concentration of deuterium is maintained in the body water. Treatment of normal female rats, maintained under the same conditions, either with adrenocorticotrophic hormone (ACTH) or with growth hormone (GH) leads to a diminished uptake of deuterium in the liver and body fats, relative to that observed in normal, untreated control animals.

Methods

All of the animals used were female albino rats of the Sprague-Dawley strain as supplied by the Holtzman Company. They weighed between 150 and 200 grams upon arrival in the laboratory, and they were maintained on a diet of Laboratory Chow until they had attained their "plateau" weights, of the order of 230 to 280 grams. Each group of animals was maintained for a week, in advance of the experiment proper, upon the fat-free high carbohydrate diet of the composition given below. During this period and the subsequent experimental period the rats were kept in individual wire-bottomed cages in a room at constant temperature (75° F.). Measurements of food intake were made at two-day intervals. Adrenalectomy was performed by the dorsal-lumbar approach two weeks before the beginning of the experiment proper. The animals were maintained thereafter on drinking water containing 0.7 per cent sodium chloride and 0.3 per cent sodium bicarbonate.

The animals receiving ACTH (Armour Lot 41-L-3) were given a total dose of 1 mg. per 100 gm. of body weight daily, in two intraperitoneal injections of 0.1 ml.

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* From the Department of Physiological Chemistry, Yale University School of Medicine. The data presented here are taken, in part, from a thesis submitted by Isaac D. Welt to the Graduate School of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The work was done during the tenure of a U.S. Public Health Service Pre-doctoral Fellowship.

† Present address: The Public Health Research Institute of the City of New York, Inc., Foot of East 15th Street, New York 9, New York.

‡ Present address: Department of Biochemistry, School of Medicine, Emory University, Georgia.

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of saline solution. Those receiving growth hormone were given intraperitoneally 1 mg. daily of a highly purified crystalline preparation (Armour, Lot 22KR2) in 0.2 ml. of saline solution. In both instances, treatment was begun 4 days before the experiment and was continued throughout the 8 days of the experiment.

The high-carbohydrate, fat-free diet was of the following percentage composition: casein, 15; dextrin, 50; cornstarch, 30; salt mixture (Phillips and Hart[17] modified by the addition of cobalt chloride), 5; plus the following vitamins (in mg. per kilogram of diet): thiamine hydrochloride, 8; riboflavin, 5; nicotinic acid, 50; pyridoxine hydrochloride, 5; calcium pantothenate, 50; biotin, 5; folic acid, 0.5; menadione (synthetic vitamin K), 5; α-tocopherol acetate, 100; choline chloride, 1000. Vitamins A and D were given separately, by medicine dropper, at weekly intervals. For the adrenalectomized animals the potassium phosphate and the potassium iodide in the salt mixture were replaced by the corresponding sodium salts. The diet was served in food cups with a certain amount of water as a binder. A separate cup full of food was kept near the cages and weighed in order to determine water losses by evaporation, so that the measurements of food intake could be corrected for this factor.

Four groups of 12 rats each (normal, adrenalectomized, ACTH-treated, and GH-treated) were subjected to the following experimental procedure. Each animal was given a subcutaneous injection of 1 ml. per 100 gm. of body weight of a 0.9 per cent solution of sodium chloride in 99.8 per cent deuterium oxide and was provided thereafter with drinking water containing 2 per cent deuterium oxide. Groups of 3 rats were sacrificed on the first, second, fourth, and eighth day after the administration of deuterium.

At the time of sacrifice, each rat was anesthetised with ether, and 5-7 ml. of blood were withdrawn from the inferior vena cava, placed in a centrifuge tube with a little powdered potassium oxalate, and centrifuged. The plasma was distilled at low temperature in vacuo and samples of the water were stored in sealed glass ampoules. These samples were used for the determination of the deuterium concentration in the body water. After the blood sample was taken, the liver was removed, blotted, weighed, and set aside for extraction of the total fatty acids. The gastrointestinal tract was then removed, and the empty carcass was weighed and prepared for extraction of the fatty acids first by flaying the carcass and cutting the skin into small bits with scissors, next by chopping the carcass into a dozen small pieces with a cleaver, and finally by homogenizing both skin and carcass together in about 45 ml. of water in a Waring blendor. The total fatty acids were isolated from both the liver and the carcass by the methods used by Schoenheimer and Rittenberg.[21]

The samples of plasma water and of fatty acids were prepared for determination of their deuterium content by combustion in an apparatus designed by Mr. Jack Graff of this laboratory.[8] The water of combustion is reduced to hydrogen gas which is collected in small sampling "pearls" fitted with a stopcock. The deuterium content of the gas was determined in a mass spectrometer fitted with a 60° tube. The instrument was calibrated against known concentrations of deuterium. Frequent checks on the calibration were made by running determinations of known samples between short series of observations on the experimental samples. Whenever an experimental sample seemed to be aberrant, the determinations of deuterium were repeated. In every instance excellent checks were obtained.

Results

A summary of the pertinent data of these experiments is presented in Table 1. The figures are the mean values obtained at each point for each group of 3 animals. An estimate of the standard deviation within the

groups of 3 animals is placed at the bottom of the columns for liver and carcass fatty acids, for body water deuterium, and for deuterium (as per cent of body water deuterium) in the liver and carcass fatty acids.

It may be noted that there is little difference in the mean body weights of the several groups and that the changes in weight during the experiment

TABLE 1

SUMMARY OF GENERAL DATA FOR THE NORMAL, ADRENALECTOMIZED, GROWTH HORMONE-TREATED, AND ACTH-TREATED FEMALE RATS*

Group	*Day*	*Final body weight (gm.) and weight change*	*Average food intake (gm. per 100 gm. body weight)*	*Fatty acids (gm. per 100 gm. body weight)*		*Body water deuterium atom per cent excess*	*Deuterium in fatty acids (as per cent of water deuterium)*	
				Liver	*Carcass*		*Liver*	*Carcass*
Normal	1	244 (+2)	8.4	0.133	8.35	1.27	15.58	2.40
	2	262 (+3)	8.0	0.130	9.4	1.29	24.53	3.20
	4	265 (+6)	7.5	0.106	9.2	1.46	20.32	4.11
	8	258 (+5)	7.9	0.126	8.6	1.36	26.03	5.72
Adrex	1	257 (+7)	8.2	0.110	6.5	1.41	17.96	3.34
	2	264 (+2)	7.2	0.106	6.4	1.31	22.50	4.13
	4	271 (—1)	5.4	0.096	7.1	1.31	24.36	5.77
	8	271 (—8)	7.4	0.108	7.2	1.40	36.40	18.34
Normal + growth hormone	1	267 (+3)	8.9	0.138	9.0	1.43	8.09	1.14
	2	261 (+5)	8.5	0.142	8.35	1.43	9.91	0.93
	4	261 (+7)	7.9	0.128	7.8	1.42	16.13	2.20
	8	258 (+10)	7.7	0.144	7.0	1.35	13.45	4.25
Normal + ACTH	1	242 (—4)	7.1	0.121	8.3	1.35	9.80	0.57
	2	244 (—1)	7.4	0.125	7.95	1.26	12.60	1.52
	4	239 (0)	8.2	0.121	8.2	1.32	15.20	3.99
	8	230 (—7)	7.8	0.104	7.9	1.18	22.79	4.45
Standard deviation within groups of 3 rats:				±0.017	±1.05	±0.09	±4.14	±2.10 (±0.94)†

* The figures are the mean values for groups of 3 rats on each day.
† Excluding adrenalectomized group on day 8.

are, on the whole, small. The growth hormone-treated animals show the expected steady gain in weight during the experiment, and the ACTH-treated animals show a slight tendency to lose weight as the experiment progresses. The quantities of liver fat are seen to be fairly uniform among all of the groups of animals, and they show no tendencies toward progressive change from day to day within each of the large groups of 12 animals. Somewhat less uniformity is seen in the quantities of carcass fats. There is a progressive decrease with time in the carcass fats of the growth-hormone treated

animals which is in accord with the effects of this hormone previously observed.[14, 15] In the other three large groups of 12 animals there is no discernible change in carcass fat with time. The adrenalectomized animals, however, have somewhat lower carcass fats than all of the others. This is due, at least in part, to the tendency of adrenalectomized animals toward a certain capriciousness of appetite. Some preliminary trials with different diets were made in an attempt to overcome this difficulty, and although the adrenalectomized animals maintained relatively good appetites on the diet described above, it is seen that the fourth-day group of adrenalectomized animals deviates markedly in food intake from all of the other groups. It is probable that the decline in carcass (and in liver) fat in the adrenalectomized animals took place in the period shortly after operation, before they recovered and stabilized on the synthetic diet. The smaller quantities of liver and carcass fats in this group affect the experiment in the sense that the dilution of deuterium incorporated in newly synthesized fatty acids is less in this group than in the others; the higher deuterium concentration could therefore give a false impression of greater synthesis. It may be said, however, that these differences in liver and carcass fats are in fact not great enough to outweigh the differences in deuterium uptake observed between the normal and the adrenalectomized rats.

A satisfactory uniformity in concentration of deuterium in the body water was achieved in all of the groups. The variation within groups of the deuterium concentration in the liver fats (expressed as per cent of the body water deuterium) was also reasonably uniform. The variation in deuterium concentration in the carcass fats was seriously aberrant in only one group (that of the adrenalectomized animals on day 8). The gross data therefore have the degree of uniformity required for reasonable confidence that the changes in deuterium concentration with time, in the different experimental groups, may be related to rates of synthesis of fatty acids. The concentrations of deuterium found in the liver and carcass fatty acids of the normal animals are very like those observed by other workers in similar experiments.[8]

It was hoped, as the design of these experiments indicates, to make an analysis of the kinetics of fat synthesis under each condition of treatment. At the same time the use of 3 animals at each point was intended to show whether the factor of biological variation (which has not been taken into account in any previous work of this kind) is in fact sufficiently small to make secure the assumptions upon which analyses of kinetics have been based.[23] These data suggest that this is not so. If one examines the data relating deuterium concentration in either liver or carcass fats to time (day 1 to day 8), for each experimental group, there is in no instance a significant departure from linearity for this relation.

The data were therefore treated by analysis of variance (see Snedecor,[22]

section 11.8, p. 275) by which it is possible to determine the significance of differences due to the different treatments over all four points of the experimental period. In this method all of the experimental data are used.

The observed variations are divided into those occurring between groups (i.e., the observed effects of treatment, of time, and of "interaction"—that is, any difference in the manner of the response to treatment over the time course of the experiment), and into the variations within groups—the random error of the observations. The ratio of the mean square for treatment to the mean square for error (F) is determined for any pair of experimental groups—i.e., normal versus adrenalectomized, ACTH-treated, or

TABLE 2

SUMMARY OF ANALYSIS OF VARIANCE:
LIVER FATTY ACID DEUTERIUM

Source of variation	*Sum of squares*	*Degrees of freedom*	*Mean square*	*F*†	*p*
Normal vs. Adrex					
Total	1176.6226	23			
Between groups*	837.3983	7			
Within groups (error)	339.2283	16	21.20		
Treatment	81.7704	1	81.77	3.86	>0.05<0.1
Normal vs. ACTH					
Total	909.2967	23			
Between groups	696.3346	7			
Within groups (error)	212.9621	16	13.31		
Treatment	240.0970	1	240.10	18.04	<<0.01
Normal vs. GH					
Total	1088.1496	23			
Between groups	880.9695	7			
Within groups (error)	207.1801	16	12.95		
Treatment	566.3845	1	566.4	43.7	<<0.01

* Variation between groups includes effects of time, treatment, and interaction. There was no significant interaction in any of these comparisons. The differences due to time were all highly significant.

† $F\ (1, 16) = 4.49$ for $p = 0.05$; $= 8.53$ for $p = 0.01$.

GH-treated animals. The magnitude of this ratio is related to the probability (p) that the differences in treatment could have occurred by chance. If the combined data of all four treatments are analyzed in the same way, it can be seen that there are significant effects of treatment, time, and interaction, but it is necessary to make the comparison in pairs, as indicated, in order to determine which of the treatments differs significantly from the normal controls.

Table 2 presents a summary of the analysis of variance for the deuterium concentration in the liver fatty acids. It is seen that treatment with either ACTH or GH brings about a significantly smaller accumulation of deuterium during the experimental period than that seen in the normal animals. The adrenalectomized animals attain higher levels of deuterium

in the liver fatty acids than the normal animals do, but the difference just fails to be significant at the 5 per cent level. From Table 1 it is seen that the observations overlap on days 1 and 2, but that the levels in the normal animals do not differ significantly after the first day, whereas those in the adrenalectomized animals increase on both the fourth and the eighth day and are certainly significantly higher than the normal values at these times.

The quantity of fatty acids in the liver at any time is a resultant of four variables: hepatic fatty acid synthesis, transport to the liver (after ingestion or from the depots), oxidation in the liver, and transport from liver to depots. The two latter processes are assumed to draw upon representative samples of the liver fatty acids, and they should not, therefore, influence the deuterium concentration. The transport of fatty acids from depots of relatively low isotope concentration to the liver can easily dilute the deuterium accumulated by even the most active synthetic process in the small fatty acid store in that organ. Since the rates of synthesis in and of transport to the liver may vary independently, no conclusions can be drawn from these data about the relative rates of synthesis of fatty acids in the livers of the normal as compared to the differently treated rats in this experiment. It is known, for instance, that adrenalectomized animals do not mobilize fats to the liver as readily, in a number of emergencies, as normal animals.[11] The observations in these experiments might therefore be accounted for if there is some retardation of fat transport to the liver in adrenalectomized animals. Both ACTH and GH have been shown to be ketogenic.[3] The smaller accumulation of deuterium in the liver fatty acids of the animals treated with these hormones might therefore be due to an increased rate of mobilization of fat to the liver. It is of course possible that each of these treatments does have an effect on hepatic fatty acid synthesis, but this cannot be concluded from the liver data above.

Table 3 presents a summary of the analysis of variance for the deuterium concentration in the carcass fatty acids. It is seen that the carcass fatty acids in the adrenalectomized animals have attained significantly higher concentrations of deuterium than those in the normal animals and that the accumulation of deuterium in the carcass fatty acids of the ACTH- and GH-treated rats is significantly lower than in the normal animals.

The quantity of carcass fatty acids at any time is also a resultant of four variables: peripheral (non-hepatic) fatty acid synthesis, transport of fat from the liver or the intestine to depots, oxidation, and transport of fat to the liver. As in the instance of the liver, the latter two processes are not expected to affect the deuterium concentration of the carcass fatty acids. Since the deuterium concentration of the liver fatty acids is always considerably higher than that of the carcass fatty acids (Table 1), transport of fat from the liver to the peripheral tissues can have the effect only

of increasing the deuterium concentration of the carcass fatty acids. Since in animals on a fat-free diet there is no process tending to dilute the deuterium of the carcass fatty acids, the concentrations of isotope found there may be taken as a measure of the amount of synthesis of fatty acids taking place during the experiment. It may be noted that on the average, for the 48 animals used in these experiments, there is 65 times as much carcass fat as liver fat. The ratio of liver fatty acid deuterium to carcass

TABLE 3

SUMMARY OF ANALYSIS OF VARIANCE: CARCASS FATTY ACID DEUTERIUM

Source of variation	*Sum of squares*	*Degrees of freedom*	*Mean square*	*F*†	*p*
Normal vs. Adrex					
Total	686.5079	23			
Between groups*	562.0007	7			
Within groups (error)	124.5072	16	7.78		
Treatment	97.8076	1	97.81	12.57	<0.01
Normal vs. ACTH					
Total	75.6048	23			
Between groups	59.3107	7			
Within groups (error)	16.2861	16	1.02		
Treatment	9.0160	1	9.02	8.84	<0.01
Normal vs. GH					
Total	65.3532	23			
Between groups	56.9398	7			
Within groups (error)	8.4134	16	0.53		
Treatment	17.9055	1	17.91	33.8	<<0.01

* Variation between groups includes effects of time, treatment, and interaction. There is significant interaction only in the comparison: normal vs. adrex, which means that the deuterium concentration in the carcass fatty acids of the adrenalectomized animals was changing in a different fashion with time. The differences due to time were all highly significant.

† $F\ (1, 16) = 4.49$ for $p = 0.05$; $= 8.53$ for $p = 0.01$.

fatty acid deuterium averages 4.5:1. There is therefore, on the average, some 14 times as much deuterium in the carcass fatty acids as there is in the liver fatty acids. The carcass fatty acids, despite their lower isotope concentration, much more nearly represent the amount of fatty acid synthesis that has occurred in the whole body during the experimental period.

The analysis in Table 3 indicates that treatment of normal female rats with ACTH or with GH significantly inhibits the process of fatty acid synthesis. The fact that the ACTH-treated rats have somewhat smaller amounts of carcass fats than the normal animals and that treatment with growth hormone brings about a progressive decline in the carcass fatty

acids, does not prejudice this interpretation, since the accumulation of isotope in the smaller pool, at the same rate as in the normal animals, would lead to a higher concentration of isotope in the carcass fatty acids of the treated animals.

Although the adrenalectomized animals have a significantly higher concentration of deuterium in the carcass fatty acids than the normal animals do, two difficulties must be met before this observation can be interpreted with confidence. First, the method of analysis of variance depends for validity upon uniformity of the variances among the groups of observations. The variance of the observations on one group of adrenalectomized animals (Table 1, day 8) differs widely from that of all of the other groups. The variance is, however, roughly proportional to the mean isotope concentration of that group of 3 animals, and it is therefore possible to control this aberration by conducting the analysis of variance in terms of the logarithms of the observed isotope concentrations in the carcass fatty acids of the normal and the adrenalectomized animals. A summary of this analysis is presented in Table 4, A. It is seen that there is still a highly significant difference.

The second difficulty is that the adrenalectomized animals as a whole have somewhat smaller amounts of carcass fatty acids than the normal animals. The observed isotope concentrations might therefore be artificially high and not related to increased synthesis of fatty acids. An attempt was made to control this "dilution effect" roughly by taking, in each instance, the product of the amount of carcass fatty acid (in gm./100 gm. final body weight) and of deuterium concentration (as per cent of body water deuterium). For the reason that the group variances differed but were roughly proportional to the group means, the analysis of variance was done with the logarithms of the products so obtained. The summary, presented in Table 4, B, shows that the difference is still comfortably above the 5 per cent level of significance.

It may be concluded, therefore, that in the albino rat on a fat-free, high-carbohydrate diet, adrenalectomy is followed by an increase in the rate of synthesis of fatty acids. Since in these experiments the amount of body fat was nearly constant during the period of observation, it follows that after adrenalectomy the rat utilizes fat at an increased rate as well. In effect the adrenalectomized animal diverts a greater proportion of carbohydrate through the metabolic pathway of fat synthesis and combustion.

Discussion

These experiments are subject to an important criticism in respect to design. Each of the experimental groups was run consecutively and not concurrently, and it may be questioned whether the responses of different groups of animals are sufficiently consistent that the same or nearly the

same result might reasonably be expected whenever the experiment is repeated. Limited time and facilities have prevented repetition of the whole experiment either in this design or in a much better design in which each experimental group contains animals under each kind of treatment and is repeated several times. The fact that the average responses of the normal rats closely resemble those observed by other workers in similar experiments and that the differences due to the different treatments are highly significant encourages confidence in the results. Nevertheless, without repetition, or independent confirmation of the experiment in a proper de-

TABLE 4

SUMMARY OF ANALYSIS OF VARIANCE:
CARCASS FATTY ACIDS
NORMAL VERSUS ADRENALECTOMIZED GROUPS

Source of variation	*Sum of squares*	*Degrees of freedom*	*Mean square*	*F**	*p*
A. In terms of logarithms of deuterium concentration.					
Total	1.634490	23			
Between groups	1.459657	7			
Within groups (error)	0.174833	16	0.0109		
Treatment	0.301056	1	0.3010	27.55	$<<0.01$
B. In terms of logarithms of products of carcass fatty acid (gm./100 gm. final body wt.) and deuterium concentrations.					
Total	1.647320	23			
Between groups	1.245530	7			
Within groups (error)	0.40179	16	0.0251		
Treatment	0.11816	1	0.1182	4.71	<0.05

* $F\ (1, 16) = 4.49$ for $p = 0.05$; $= 8.53$ for $p = 0.01$.

sign, the conclusions, however reasonable and suggestive, must be regarded as tentative.

The effect of adrenalectomy in accelerating fat synthesis and of treatment with ACTH in retarding it may come as a surprise. There are numerous references in the literature suggesting that adrenalectomized animals tend to lose their body fat.[1] Most of the recorded observations have been made upon adrenalectomized animals in a critical state of adrenal insufficiency, after a more or less prolonged period of partial inanition. Account was not taken of the marked loss of appetite that occurs in animals in adrenal insufficiency. More recently, Schiffer and Wertheimer[20] have presented data in which the factor of food intake is taken into account. They observed that the fat content of adrenalectomized rats was much less than that of pair-fed controls after a period of several days on the same diet. The composition of the diet and the level of food intake were

not recorded. The amounts of fat present were measured by the dissection and weighing of obvious fat depots rather than by extraction of total fat or fatty acids from the comminuted whole carcass. The amounts of fat present in the bodies of representative groups of experimental animals at the beginning of the experimental period were not observed or recorded. One cannot therefore know what normal variations in body fat occur among this particular strain of rats and what *changes* in amount of fat might have taken place during the experiment. The insufficient method of analysis also makes the interpretation difficult. It may be noted that one conclusion of the present experiments is that the rate of fat utilization, as well as that of fat synthesis, is increased after adrenalectomy, since the adrenalectomized animals showed no tendency to gain body fat during the experiment. If appetite and food intake are not maintained by providing a diet pleasing to the animals and by maintaining them on extra sodium chloride, it may be expected that adrenalectomized animals in a state of partial inanition will indeed lose their body fat at a rate greater than normal. Bernhard and Steinhauser[4] have presented good evidence illustrating the marked effect of inanition in depressing fat synthesis. The demonstration that this process is accelerated in adrenalectomized rats therefore depends closely upon finding conditions in which the level of food intake is at or near the normal level. Such conditions were, on the whole, satisfactorily met in these experiments.

There have been few studies of the effects of ACTH on fat metabolism in the rat. Evans and his colleagues[1] have shown that this hormone is ketogenic, which invites the suggestion that ACTH may increase fat utilization. Li, Evans, and Simpson[15] have also reported that treatment with ACTH appears to increase the fat content of hypophysectomized rats. In their experiments, only the final body composition of the animals was reported, and the comparisons were made between untreated hypophysectomized rats and similar animals treated for ten days with ACTH. The food intakes were kept constant by arbitrary restriction to 4.5 gm./day. Again, since the initial body composition of a representative group of the experimental animals was not reported, it is difficult to interpret these observations. Differences in the composition of the weight loss during the experiment might account for the results observed. Ingle[13] has observed that in normal rats force-fed a constant daily quantity of a high-carbohydrate diet and subjected to continuous subcutaneous injection with ACTH for a period of three weeks, the body fat disappears almost completely. This observation is consistent with those of the present experiments.

The observation that adrenalectomized animals tend to use a greater proportion of their carbohydrate over the pathway of synthesis and subsequent oxidation of fat and that this tendency is opposed by ACTH may

help to throw light upon a number of other phenomena. It is well established that adrenalectomized animals lose their liver glycogen store during fasting at a greater than normal rate. They are also unusually sensitive to insulin, a hormone shown by Stetten and his colleagues to have a marked accelerating effect on the synthesis of fat from carbohydrate.[25, 26] Adrenalectomy, as well as hypophysectomy, leads to an amelioration of pancreatic diabetes.[16] That this is not due only to a decrease in the processes of protein catabolism and gluconeogenesis is indicated by the facts that in such doubly operated animals the D:N ratio falls to very low levels (indicating an actual increase in carbohydrate utilization) and that the administration of adrenal cortical extract may lead, in the fed animal, to an increased sugar output rather in excess of that which can be accounted for by the concomitant increase in nitrogen excretion. Stetten and Boxer[24] have shown that in the diabetic rat on a high-carbohydrate diet the process of conversion of sugar to fat is very nearly completely suppressed. It may be suggested that the removal of the hypophysis or of the adrenals relieves an inhibition of fat synthesis and that in the hypophysectomized-diabetic or adrenalectomized-diabetic animal one has a preparation in which the process of conversion of carbohydrate to fat is resumed at its "natural" rate, unregulated by the endocrines, and subject mainly to variations in the amount and composition of the diet and, possibly in some small degree, to fluctuations in immediate metabolic requirements for one or the other of the principal energy-yielding substrates.

An aspect of the "diabetogenic" action of the adrenal cortical hormones is illustrated by the work of Ingle.[13] In his experiments normal rats, daily force-fed a constant large amount of a high carbohydrate diet, exhibited a glycosuria of considerable magnitude during periods in which they were injected with cortical extracts or with crystalline cortical hormones. The concomitant increase in daily nitrogen excretion was far too small to account for the amounts of sugar excreted. The suggestion is strong that these animals, under the influence of cortical hormone, were no longer able to dispose of the large amounts of carbohydrate that they dealt with easily both before and after the periods of hormone injection. From these experiments and the observations reported here, it may be suggested that part, at least, of the "diabetogenic" action of the adrenal cortical hormones (and of ACTH) can be ascribed to their property of inhibiting the process of conversion of carbohydrate to fat.

The action of growth hormone in inhibiting the process of fat synthesis is of very great interest in the light of the demonstration by Young and his colleagues,[7] by Houssay and Anderson,[9] and by Campbell and his colleagues[6] that highly purified growth hormone is diabetogenic in the intact cat and dog. In this laboratory Russell[19] has also observed that large doses of purified growth hormone can intensify the mild diabetes of alloxan-

treated rats. The "glycostatic" actions of anterior pituitary extracts,[18] which can be reproduced by purified growth hormone,[10] indicate that this agent plays an important part in the conservation of carbohydrate by the organism. The glycostatic action of growth hormone in the fasting hypophysectomized rat is independent of the presence of the adrenal cortex,[2] but the glycostatic action of this hormone in the fasted rat given a glucose meal cannot be obtained in the adrenalectomized rat unless a dose of adrenal cortical extract, too small to have any effect in itself, is administered along with the growth hormone.[18] Since the present experiments were conducted on intact animals, it is impossible to learn from them what respective parts the adrenal cortical hormones and the growth hormone play in suppressing liponeogenesis. The interesting questions are raised, however, whether the growth hormone is the primary agent and the cortical hormone is necessary but merely permissive (as for the expression of the glycostatic action in glucose-fed rats), or whether the cortical hormone is the primary agent, but requires a minimal amount of growth hormone for the complete expression of its effect, or whether each has independent effects, possibly on different sites of fat synthesis in the body.

The implications of the experiments reported here which have been developed in the foregoing paragraphs are subject to experimental examination. It is hoped that additional work on these lines can be carried out in the future.

Summary

An attempt has been made to study certain endocrine influences on the process of liponeogenesis from carbohydrate by measuring the uptake of deuterium into the liver and carcass fats of plateaued female rats on a high-carbohydrate, fat-free diet. Four groups of 12 animals each were used: normal rats; adrenalectomized; normal, treated with adrenocorticotrophic hormone; and normal, treated with growth hormone. The animals were given deuterium oxide equal to 2 per cent of their body weight and were provided with drinking water containing 2 per cent deuterium oxide during the eight days of the experiment. Groups of 3 rats were sacrificed on the first, second, fourth, and eighth day after administering deuterium, and the deuterium content of the body (plasma) water and of the isolated liver and carcass fatty acids was determined.

It is found that the amount of deuterium taken up into the liver and carcass fats of the adrenalectomized animals is significantly greater than that taken up into the liver and carcass fats of the normal rats. Treatment with both ACTH and growth hormone results in a significantly smaller uptake of deuterium into the liver and carcass fats.

It is concluded that adrenalectomy is followed by an increased rate of liponeogenesis from carbohydrate and that the adrenalectomized animal

tends to utilize a greater proportion of carbohydrate over the pathway of fat synthesis and oxidation. Conversely, activation of the adrenal cortex, or administration of growth hormone (which may be accompanied by increased adrenal cortical activity or require at least the presence of an active adrenal cortex) powerfully inhibits the process of fat synthesis from carbohydrate.

The bearing of these results upon other known aspects of the endocrine regulation of carbohydrate metabolism is discussed.

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INTRA-ARTERIAL PRESSURE MEASUREMENT WITH STRAIN GAUGE AND AMPLIFIER*

SAMUEL GELFAN AND ATTIE YVONNE WERNER

The purpose of this paper is briefly to describe intra-arterial pressure recording by means of a strain gauge pressure transducer† and amplifiers. The use of resistance wire strain gauges for blood pressure recording with galvanometers has already been described.[1,3] The use of the strain gauge with electronic amplification as a plethysmograph has also been described.[2] Since alternating input current may be used to energize these transmitters, we found it possible to register blood pressure along with other variables on a multichannel recorder with conventional condenser coupled amplifiers. The blood pressure records thus obtained, as shown in Figure 1, consist of bands or ribbons formed by sine-wave oscillating current, rather than single lines. The whole envelope of the record represents the pressure (the increase and decrease of the latter indicated by the broadening and narrowing of the envelope), and the pulse pressure curve is bilaterally symmetrical. It is only when the pressure is zero, as indicated in the calibration, that the envelope constricts bilaterally to a line. The choice of frequency of input current is determined principally by the frequency responses of the registering instrument. Thus, in the Grass electroencephalographic unit we found 50 cycles per second as the most convenient frequency. In the Sanborn cardiette the frequency response of the galvanometer limits the frequency input to an optimum of 100 cycles per second. If it is desirable to use a cathode ray oscillograph, the frequency can obviously be much higher. As may be seen in *B* and *C* portions of Figure 1, the sine-wave oscillations may serve, in addition, as small-time interval markers. Calibration of the system with a mercury manometer shows a linear relationship in the range of 0-150 mm. Hg.

For the registration of systolic and diastolic pressures the precaution against bubbles in the liquid system is well known. This was particularly important in our experiments in which the animal and strain gauge were subjected to partial vacuums in order to simulate high altitudes, where bubbles that may be microscopic in size expand enormously with marked reduction in atmospheric pressure. We therefore used thick walled transparent polyethylene tubing (5.5 mm. o.d., 2 mm. i.d.) instead of lead to facilitate the visual detection of bubbles in the entire system. This tubing

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† Model P23-8-350, manufactured by Statham Laboratories.

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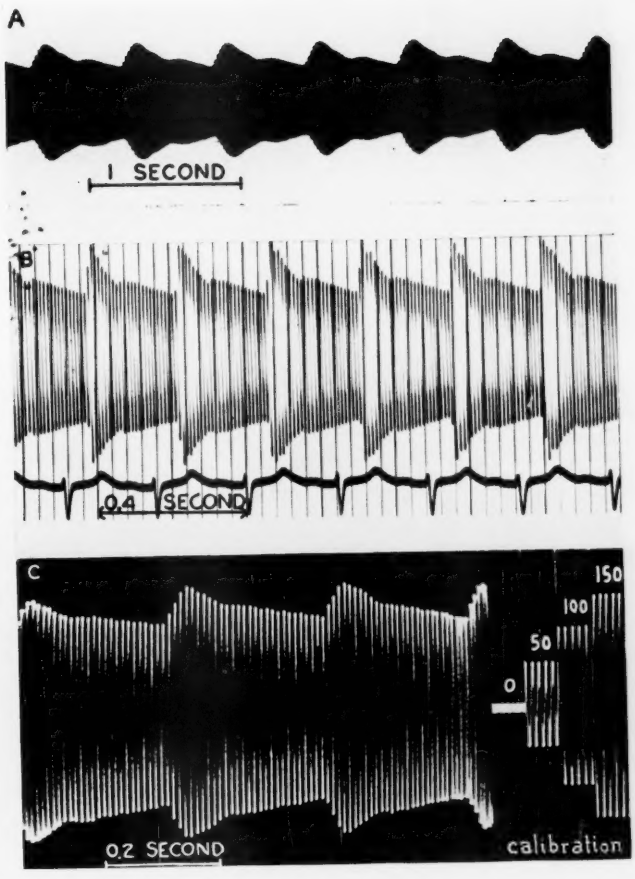

FIG. 1. Intra-arterial pressure measurements with strain gauge transmitter.

A. Blood pressure in man following hyperventilation, recorded on Grass electroencephalograph: 18-gauge hyperdermic needle in brachial artery and 14 cm. polyethylene tubing connecting it with strain gauge transmitter; 60 cycles/sec. input current.

B. Blood pressure in rhesus monkey, anesthetized with Nembutal, recorded on Sanborn cardiette: 14 cm. polyethylene tubing drawn out at one end (equal to 18-gauge needle) connecting femoral artery with strain gauge transmitter; 100 cycles/sec. input current. Lower portion of record = ECG, unipolar VC lead.

C. Same as *B*, on another rhesus monkey, recorded on cathode ray oscillograph. Calibration on right, with mercury manometer. All records about ⅔ of originals.

connects the artery of the animal with the pressure head of the transmitter. We found it convenient to draw out the end of the polyethylene tubing for femoral artery cannulation, thus eliminating the hypodermic needle in our work on small monkeys. For larger animals, where arterial puncture may be effected through the skin with a hypodermic needle, the latter is appropriately attached to the polyethylene tubing. At sea level, this method of blood pressure recording has been satisfactorily used also in cats, dogs, and man.

The "natural frequency" of our system, i.e., the free oscillatory response following the sudden reduction of an imposed pressure, was 55 per second. It should be emphasized that the term "natural frequency" applies to the fluid filled system comprising this model of strain gauge and the 14 cm. long polyethylene tubing drawn out at the end to a point equal to an 18-gauge hypodermic needle. The fluid in the pressure head of the gauge was 5 per cent sodium citrate, and the polyethylene cannula was filled with undiluted liquid heparin. For accurate measurements of systolic and diastolic blood pressures, particularly during marked alterations of these pressures and marked variations in heart rate, it is important to determine the effect of variations in frequency or rate of pressure changes on the relative sensitivity of this recording system, i.e., the over-all frequency response curve.* This was done by means of a very small piston cylinder arrangement which replaced the animal artery as a source of pressure, the rest of the system remaining the same as in the experiments—all of it, including the metal cylinder, filled with fluid. The motor driven piston varied the pressure in this system sinusoidally from zero up to a set level (usually 100 mm. Hg) at frequencies up to 66 cycles per second, the upper limit of frequency change that was possible to impose by this testing arrangement. Such determinations with varying rates of pressure change indicated that the sensitivity of the transducer and associated equipment used in the experiments does not alter significantly with pressure change frequencies up to 66 cycles per second. In all calibrations the input current to the strain gauge transducer, as in the experiments, was supplied by a sine-wave signal generator; the frequency usually used was 100 cycles per second.

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OBSERVATIONS ON CIRCULATING ADRENAL CORTICAL STEROIDS AND THEIR INACTIVATION *IN VITRO**

JOSEPH D. FELDMAN†

Vogt[8] in 1943 reported a life-sustaining principle in the blood of the adrenal vein of a variety of animals which was not present in right heart or arterial blood. Schrek[2] in 1949 described a cytotoxic action on lymphocytes *in vitro* of microgram quantities of several adrenal extracts and pure crystalline cortical steroids. Feldman[1] found a rapid and marked destructive action of Lipo-Adrenal Cortex (Upjohn) and of 11-dehydrocorticosterone acetate (Compound A) on several different cell types.

The present work was undertaken to attempt to assay circulating adrenal steroids by *in vitro* methods. It was postulated that these hormones would destroy lymphocytes, and the number of cells lost from an accurately counted suspension would be proportional to the effective cortical steroids in plasma or serum. Such a method to detect these compounds in peripheral blood would be of use clinically in determining the function of both the adrenal and pituitary glands. Despite the negative results of the investigation, this work is being presented because of the interest it may have for others in the field. In addition, the data on *in vitro* inactivation of adrenal steroids is offered as a possible explanation for failure to detect circulating cortical steroids.

Material and methods

Rats of the Sprague-Dawley or Rockland Farm strain were used for all experiments, except two in which dogs were employed. The rats ranged in age from 60 to 200 days. Three different means were employed in an attempt to increase adrenal steroids in the blood to detectable levels.

1. Stress. Stress was accomplished in the following ways: (a) forced swimming for 1 to 3½ hours; (b) exposure to cold for 18 hours; (c) acute hemorrhage; (d) restriction of motion by tying the animal to a board. From a few minutes to 4 hours following the stress the animals were bled from the heart under light ether anesthesia.

2. Injection of cortical extracts. Lipo-Adrenal Cortex (Upjohn) was given in doses of from 1 to 3 cc., always subcutaneously. Two aqueous cortical extracts (Upjohn and Wilson) were injected either subcutaneously or intraperitoneally in doses ranging from 1 to 8 cc. Three to 12 hours after the injection the animal was bled from the heart.

3. Injection of ACTH and Adrenalin. ACTH (Armour) was dissolved in physiological saline to a concentration of 7 mgm. per cc. One cc. of this solution was administered subcutaneously for three injections at 8-hour intervals. The animal was

* From the Department of Pathology, Yale University School of Medicine. This investigation was carried on during the tenure of a post-doctorate research fellowship from the U. S. Public Health Service.

† Present address: Hadassah Medical School, Hebrew University, Jerusalem, Israel.

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bled from the heart 8 hours after the final dose, or 24 hours following the initial dose. Adrenalin (Sharp and Dohme) in doses of 100 μg. per kilogram was injected subcutaneously and cardiac blood was collected between 1 to 6 hours after the injection.

The cardiac blood was heparinized with 0.2 ml. of Liquaemin (Roche-Organon, 10 mgm. of heparin per cc.) and then centrifuged for 15 minutes at 3000 r.p.m. The plasma was withdrawn and in it fragments of thymus of the same stressed animal were teased and the resulting suspension filtered through 80-gauge monel wire gauze. The final number of cells was adjusted by addition of plasma.

A baseline or zero hour count was done on these suspensions within 30 minutes of preparation. At varying intervals thereafter counts were made to determine the total number of cells and the number of non-viable cells by the method previously described.[1] From these counts the rate of cell lysis and the rate of cell death could be determined.

In each experiment a control rat was used and its blood removed and handled as described above. Control animals for swim or cold stress rats were kept in cages at room temperature (25-26 degrees C.). Control animals for cortical extract, ACTH, or Adrenalin received cottonseed oil or saline by the same route and in the same dosages as was given to the experimental rats.

To learn whether cortical steroids of adrenal glands could be detected and quantitated, sliced adrenal tissue was incubated with cell suspensions of known number and counts were done as described above. For controls, sliced kidney or muscle was incubated with the same cell suspension used for the adrenal glands. A baseline count was done within 45 minutes of the death of the animal as previously described.[1] Cell suspension plus adrenal tissue and cell suspension plus kidney or muscle tissue were incubated at 37.5 degrees C. or at room temperature (25-26 degrees C.) with continuous shaking.

It was conjectured that ACTH might release cortical steroids from adrenal glands *in vitro*. Accordingly, in one series of tests ACTH was added to a plasma suspension, to the same plasma suspension containing sliced adrenal tissue, and to the suspension containing sliced renal tissue as a control. The ACTH was prepared by dissolving 15 mgm. in 1 cc. of Gey's medium* and 0.2 cc. of this solution (3 mgm. of ACTH) was added to the suspensions.

The adrenal veins of two heparinized, anesthetized (Nembutal) dogs were cannulated according to the technique of Vogt[3] and from the separated plasma and homologous lymph nodes suspensions of lymphocytes were made.

For *in vitro* inactivation studies the following procedure was employed. An unstressed male rat was bled from the heart; the blood was heparinized as above, and a suspension of lymphocytes was made from the thymus of the same animal. The number of lymphocytes in suspension ranged from 20,000 to 40,000 cells per cu. mm.; 0.4 ml. of this suspension was used to test the toxic action of Lipo-Adrenal Cortex, as described previously.[1]

Fragments of muscle, intestinal tract, thymus, adrenal, kidney, and brain tissues were placed in separate vials, each of which contained 0.1 ml. of Lipo-Adrenal Cortex (4 rat units) plus 0.3 to 0.4 ml. of cottonseed oil with 1% chlorbutanol. In previous

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* Gey's Medium

NaCl	8.000 gm.
KCl	0.372 "
$NaHCO_3$	0.500 "
$CaCl_2$	0.203 "
$MgCl_2(6H_2O)$	0.209 "
$Na_2HPO_4(2H_2O)$	0.143 "
KH_2PO_4	0.052 "
Dextrose, anhydrous	1.000 "
H_2O	1000.00 cc.

experiments it was found that 0.8 rat units of this extract produced 90% or more non-viable cells within 12 hours of incubation when added to lymphocyte suspensions.[1] Cottonseed oil with 1% chlorbutanol, by itself, produced less than 20% non-viable cells in 12 hours.[1] The fragments of tissue and the extract were shaken together for 3 hours, 150 times per minute, at room temperature. The tissues were then removed and to the remaining Lipo-Adrenal Cortex 0.4 ml. of the test lymphocyte suspension was added. Control vials containing cottonseed oil with 1% chlorbutanol and the Lipo-Adrenal Cortex were shaken in the same manner without addition of tissue fragments.

A count was made on the mixture of adrenal extract and lymphocyte suspension after 5 minutes of shaking and this was regarded as the baseline, or zero hour, determination. At the same time the number of non-viable cells was ascertained and calculated as a percentage of the total number. Subsequent counts were done at 6 to 12 hours of incubation to determine the number of non-viable cells according to the method previously described.[1]

Results

As reported elsewhere,[1] lymphocytes suspended in plasma and incubated at 37.5 degrees C. showed a rise in non-viable cells from 1.5% at the baseline count to 21% at 12 hours; at room temperature the rise was from 1.5% at 0 hours to 8.5% at 12 hours. In addition, the total count increased, remained constant, or decreased slightly depending on whether there was cell proliferation or not. Lymphocyte suspensions to which cottonseed oil containing 1% chlorbutanol had been added yielded results similar to those found in simple plasma suspensions. In contrast, lymphocyte suspensions mixed with Lipo-Adrenal Cortex showed 60% non-viable cells at the baseline count and 100% at 12 hours of incubation.

Suspensions made from plasma of stressed animals, of animals injected with cortical extracts, Adrenalin, or ACTH, showed the same increases in non-viable cells over the 12-hour period of observation as was found in the control suspensions. There were no statistically significant differences between the experimental and control plasmas, either at 37.5 or 25 degrees C. The interval of time which elapsed following stress or injection of extract, ACTH, or Adrenalin had no effect on the rate of non-viability and cell loss (Fig. 1).

At room temperature or at 37.5 degrees C., cell suspensions containing sliced adrenal tissue showed no significant differences from cell suspensions without the presence of tissue or with renal tissue (Fig. 1). Nor did the addition of ACTH *in vitro* cause a release of detectable steroids.

Adrenal vein plasma of dogs had no effect on homologous lymphocytes at 25 or 37.5 degrees C. compared with controls.

Of all the tissues shaken with Lipo-Adrenal Cortex only brain tissue inactivated the extract so that the test suspension of lymphocytes showed no increase of non-viable cells beyond the control mixtures. Figure 2 reveals that within 12 hours four rat units of extract produced non-viability in better than 90% of the cells after the extract was shaken with fragments of muscle, intestinal tract, lymphoid, adrenal, and renal tissue. In striking

contrast, four rat units of extract which had been shaken with brain tissue had only a slight and questionably significant effect on the cell suspension.

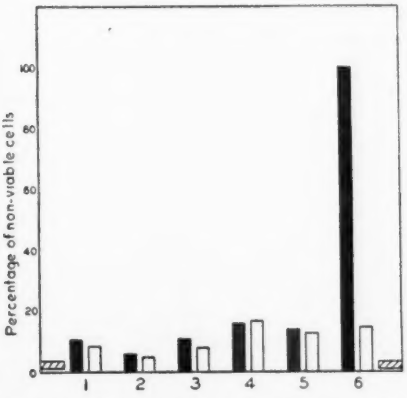

FIG. 1. Horizontal hatched bar represents the percentile range of non-viable cells at 0 hours for all mixtures. Black vertical columns show percentage of non-viability at 12 hours in experimental mixtures; white columns are control suspensions. 1. Incubation of stressed plasma and cells. 2. Plasma suspensions derived from animals injected with adrenal cortical extracts. 3. Animals injected with ACTH and adrenalin. 4. Incubation of stressed adrenal tissue with suspension. 5. Incubation of unstressed adrenal tissue with suspension. 6. Incubation of Lipo-Adrenal Cortex with suspension.

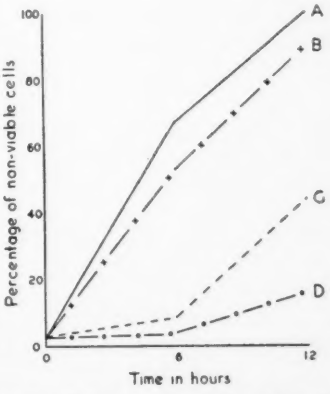

FIG. 2. Inactivation of Lipo-Adrenal Cortex. A. Incubation of Lipo-Adrenal Cortex with lymphocyte suspension. B. Incubation of Lipo-Adrenal Cortex and tissues other than brain with suspension. C. Incubation of Lipo-Adrenal Cortex and brain tissue with suspension. D. Incubation of cottonseed oil containing 1% chlorbutanol and suspension.

Discussion

Failure to detect adrenal cortical steroids in plasma or in adrenal tissue of rats subjected to various forms of stress may be due to any one or more of the following: (1) the method of stained cell counts is too insensitive to detect the minute amounts of the active hormones that may be present; (2) adrenal steroids are quickly inactivated and cannot be found in heart blood; (3) the lympholytic agent or agents of the adrenal gland are different from the life sustaining principle which was found by Vogt.[8]

The sensitivity of the method used here was previously found to be capable of responding to 0.8 of a rat unit of Lipo-Adrenal Cortex and to 80μg. of 11-dehydrocorticosterone. The amount of cortical steroid emanating from the adrenal and circulating in the peripheral blood has not been directly and quantitatively determined.

Vogt reported adrenal steroids in the adrenal vein blood and nowhere else.[8] With the present method, using adrenal vein plasma from dogs, no effects on lymphocytes were found. It is possible that the Nembutal

anesthesia employed here depressed adrenal activity. Vogt used ether and chloralose on her animals.

The *in vitro* experiments indicate that brain tissue destroys the lympholytic action of Lipo-Adrenal Cortex. Whether this is due to the high content in brain tissue of lipids which might remove the active reagents from the extract has not been determined.

Conclusions

Schrek's method was employed to study viability and cell death of cells in suspension. With this method it was found that Lipo-Adrenal Cortex had a rapid destructive action upon lymphocytes. Adrenal cortical steroids were not detected in the plasma or in adrenal glands of stressed rats or of rats injected with adrenal cortical extracts, ACTH or Adrenalin. Nor was any lympholytic activity found in the adrenal veins of two dogs with this method.

The cytotoxic action of Lipo-Adrenal Cortex was rapidly and almost completely inactivated by brain tissue but not by any other tissues tested.

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TREATMENT OF THE TYPHOID CARRIER STATE WITH CHLORAMPHENICOL*

THOMAS TIMOTHY CROCKER† AND ABRAHAM GELPERIN

The efficacy of chloramphenicol‡ in the treatment of acute typhoid fever is well established.[3, 4, 9, 10, 12, 13] However, treatment of the typhoid carrier state with this antibiotic, for varying periods of time and at dosage levels generally accepted as high, has been unsatisfactory.[2, 8, 12] Untoward reactions have also been reported,[1, 2, 5, 6] of which anorexia, nausea, and a bitter taste in the mouth were the most frequent manifestations, although occasionally glossitis and cheilosis occurred in persons under prolonged treatment with large doses of the antibiotic. This communication presents the results of a quantitative study of the carrier state and the influence of chloramphenicol upon it in two chronic typhoid carriers. In addition, a description of the development and treatment of untoward oral manifestations similar to those previously described is included.

Both subjects of this investigation had a history of acute typhoid fever (patient E. G., 24 years before, and patient R. L., 48 years before this study) and both had been known typhoid carriers for eight years. The following are pertinent data concerning these patients:

E. G., female, age 48 years, weight 257 pounds, gave a history of prolonged intolerance to fatty and fried foods and mild chronic epigastric distress and flatulence, suggestive of chronic cholecystitis. There were no episodes of jaundice, biliary colic, or acute cholecystitis. The patient's diet was interpreted as substandard with respect to animal protein and vitamin B content.

R. L., female, age 72 years, weight 183 pounds, had moderately severe cardiovascular disease of uncertain duration and was under clinic care. There was no history suggestive of biliary tract disease. The patient's diet was interpreted as barely adequate with respect to animal protein and vitamin B content.

Careful initial physical examination of both patients showed no evidence of vitamin deficiency. Repeated urine cultures were negative for *S. typhosa*. The gallbladder could not be visualized with radio-opaque medium in either patient; cholelithiasis was not evident on x-ray examination.

Methods and materials

Preliminary to chloramphenicol therapy the feces of both patients were examined bacteriologically once weekly for 14 weeks in an effort to determine quantitative

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* From the Departments of Internal Medicine and Public Health, Yale University School of Medicine, and the New Haven Department of Health.

† Hooper Foundation, University of California Medical School, San Francisco 22, California.

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variations in excretion of *S. typhosa*.* This examination continued at weekly intervals for one month during and after treatment to ascertain the extent and duration of any suppression of typhoid organisms which might result.

Samples of bile obtained by duodenal intubation before and during chloramphenicol therapy were also examined bacteriologically. At each intubation 5 to 20 ml. volumes of duodenal contents were aspirated at 5-, 15-, and 25-minute intervals after delivery into the duodenum of 60 ml. of a 50 per cent magnesium sulfate solution. All samples were examined bacteriologically and were assayed for the presence of active chloramphenicol.

Counts of *S. typhosa* in fresh stool and bile were determined by inoculating liquid Difco bismuth sulfite with 10-fold dilutions of feces or bile. Each dilution was delivered in 4.5 ml. quantities into each of two petri dishes, and 20 ml. of liquid bismuth sulfite agar were then added and mixed well by swirling. The black *Salmonella* colonies were counted after incubation for 48 hours at 37° C., and the highest dilution of inoculum which produced one to thirty colonies was recorded as the end point. Colonies from these end-point plates were identified periodically by phage typing, cultural studies, and slide agglutination tests.

Sensitivity of the *S. typhosa* strains isolated from these patients was determined with respect to chloramphenicol by two techniques. In one, an 18-hour broth culture of the organism was streaked on plates of nutrient agar containing 1.56, 2.56, 3.12, 12.5, and 25.0 micrograms/ml. of crystalline chloramphenicol. In the second technique, an 18-hour broth culture of freshly isolated *S. typhosa* was diluted to 10^{-8} and added in 1.5 ml. portions to tubes containing varying concentrations of crystalline antibiotic in 0.5 ml. of broth, so that the final concentrations of chloramphenicol were 1.87, 2.33, 3.12, 4.67, 6.25, 9.4, and 12.5 micrograms/ml. The lowest concentration of antibiotic at which no growth was visible after incubation for 24 hours at 37° C. was recorded as the level of sensitivity of the organism by the method employed.

The quantity of chloramphenicol in serum, bile, and urine was determined periodically during treatment. A modification† of the method of Joslyn and Galbraith[7, 11] was employed, in which an 18-hour broth culture of *S. sonnei* diluted to 1.5 by 10^{-8} in broth was added in 1.5 ml. volumes to samples of body fluids which had been diluted serially in broth and transferred to the culture tubes in 0.5 ml. quantities. Inhibition of growth of the test organism by the body fluids was determined visually after incubation for 18 hours at 30° C. Identical inocula of the diluted *S. sonnei* culture were added to a series of tubes containing 0.5 ml. volumes of crystalline chloramphenicol in broth in quantities such that the final concentrations of the antibiotic were 0.233, 0.312, 0.467, 0.625, 0.940, and 1.25 micrograms/ml. The quantity of chloramphenicol in each sample of body fluid was then determined by multiplying the lowest dilution of the sample that caused complete inhibition of *S. sonnei* by the number of micrograms per milliliter of chloramphenicol in the first tube of the standard series in which complete inhibition was observed. All dilutions of body fluids were prepared in duplicate, and as many samples as possible were examined in a single test. On a few occasions the same sample of body fluid was

* We are indebted to Patrick E. Bransfield, Director, and Miss Betty Hinchcliff, Technician, of the Bureau of Laboratories, New Haven Department of Health, for bacteriological studies of stool and bile samples. Cultural identification and phage typing of the *S. typhosa* strains studied here were made at the U.S.P.H.S. Communicable Disease Center, Enteric Bacteriology Laboratory, Chamblee, Georgia, and at the Connecticut State Health Department Laboratories, Hartford, Connecticut. The findings of the two laboratories were in agreement.

† Suggested by the Department of Clinical Investigation of Parke, Davis and Company, to which we are also indebted for the reference strain of *Shigella sonnei* (PD #04628) used in these determinations.

examined on two or more separate test days. No significant variation was noted among results of several tests on a single sample, nor between duplicate samples in a single test. Samples of urine and bile were filtered through Seitz pads or sintered glass if preliminary culture proved them to be contaminated. All samples were stored at —20° C. soon after being obtained from the patients.

Chloramphenicol was administered in gelatin capsules in a total daily dose of 3.75 grams, given on a schedule of 0.75 gram every four hours for five doses, in a program of self-administration. Treatment continued for 15 days (R.L.) and 19 days (E.G.), with certain departures from the original dosage schedule required by the appearance of untoward reactions, presumably due to the antibiotic. Thus, patient E.G. followed the 3.75 gram per day schedule with one 30-hour lapse during the sixth and seventh days of treatment; and patient R. L. adhered to the initial schedule until the tenth day, at which time the dosage was reduced to 2.25 grams a day. In the treatment of the latter patient the dosage was further reduced to 2.0

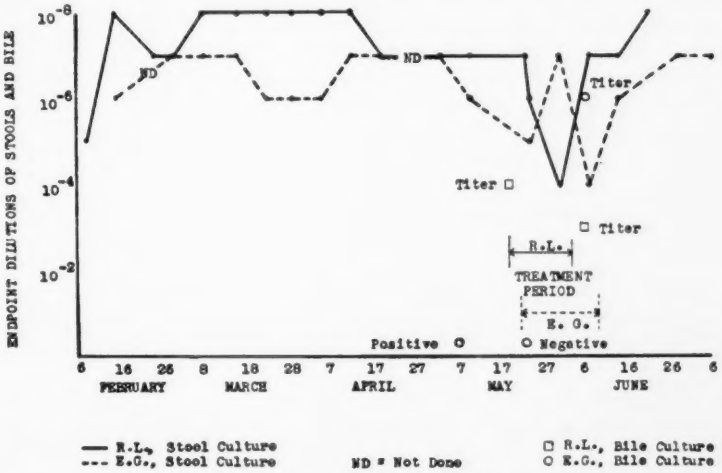

FIG. 1. Results of quantitative determinations of *S. typhosa* in samples of feces and bile obtained from two typhoid carriers before, during, and after chloramphenicol therapy.

grams a day from the twelfth to the fifteenth day of treatment. Even with the reduced dosage, the schedule of five daily doses at 4-hour intervals was retained. In terms of body weight these dosages represented 33 mg./kg./day for 19 days (E.G.); and 44 mg./kg./day for 9 days, 26 mg./kg./day for 2 days, and 23 mg./kg./day for 4 days (R.L.).

Results

Salmonella typhosa, Vi form, bacteriophage types E_1 (patient E.G.) and F_2 (patient R.L.), were consistently isolated from fecal and bile samples.

The concentrations of typhoid bacilli in fecal samples, summarized in Figure 1, were generally high and fluctuated over a ten-fold range (R.L.) or a hundred-fold range (E.G.) during the pretreatment study of each patient. An exception is evident in the first stool sample from E. G., which was negative, probably through technical error.

During chloramphenicol administration, the *S. typhosa* counts in stool specimens from R. L. on one occasion dropped one thousand-fold below the counts observed during the immediate pretreatment period. Only a ten-fold decrease in numbers of *S. typhosa* was observed in bile obtained during treatment. Excretion of *S. typhosa* in the stools of this patient returned to pretreatment levels immediately after cessation of therapy.

TABLE 1

CHLORAMPHENICOL LEVELS IN SERUM, URINE, AND BILE DURING TREATMENT OF TWO TYPHOID CARRIERS

Patient	*Day of treatment*	*Hours since last dose*	*Chloramphenicol level, gamma/ml.*		
			Serum	*Urine*	*Bile*
E.G.	0	No drug	0	0	0
	2	8	1.87	74.7	
	15	5	7.50	37.6	0 to 2.5
	16	2¾	11.28		
	19	2¾	3.76		
R.L.	0	No drug	1.87*	0	0
	2	8	7.47	74.7	
	15	4			0

* Minimal but definite inhibition of growth of the test organism was consistently produced by this serum sample although no antibiotic had been administered.

The concentration of typhoid bacilli observed in the feces of patient E. G. during antibiotic administration was depressed briefly only ten-fold below the lowest pretreatment level. One sample of bile showed considerable numbers of organisms during the treatment period.

Serum and urine levels of chloramphenicol were, in general, at the lower limit of the range to be expected from the dosage used in this trial (Table 1). The antibiotic was present in amounts varying from 1.87 to 7.4 micrograms/ml. in serum obtained 8 hours after completion of one day of treatment. This should represent the lowest serum level occurring in any 24-hour period during treatment. Chloramphenicol content of bile varied from none to 2.5 micrograms/ml. in patient E. G., and was zero in patient R. L. All specimens of bile obtained at each intubation were tested.

Sensitivity of the *S. typhosa* strains studied in this trial varied according to the technique employed. On agar plates containing chloramphenicol the limit of sensitivity was 3.12 micrograms/ml.; in broth tubes containing

chloramphenicol it was 1.87 micrograms/ml. No alteration in sensitivity of either strain of *S. typhosa* was observed by these methods before, during, or after therapy.

Untoward oral reaction. Intense pain and swelling of the oral mucous membranes, with dysphagia, rawness, burning of the tongue, and bitter tasting saliva, accompanied by anorexia, occurred in both patients during chloramphenicol administration. The symptoms of these reactions were first noted on the fifth day (E.G.) and the seventh day (R.L.) of therapy. In both patients the tongue was beefy red, with smooth lateral borders and hypertrophied papillae. Examination also revealed edema and marked reddening of the soft palate and buccal and pharyngeal mucosae. Both patients developed cheilosis of one corner of the mouth soon after the first signs appeared. Mucosal ulcerations or exudate were absent and the gums were not involved. Neither patient demonstrated skin lesions, tendon reflex changes, alterations in number or character of red or white blood cells, urinary abnormalities, or diarrhea. In patient E. G. there was an initial low fever, cervical adenopathy, and elevation of the white blood cell count. No pathogens were found in bacteriological cultures of scrapings from this patient's oral and pharyngeal mucosa, but numerous fusospirochaetal organisms were discovered on microscopic examination of a stained smear. Penicillin, 300,000 units a day for two days, administered parenterally, effected prompt relief of fever, cervical adenitis, and pharyngeal soreness, but did not alter the red, painful lingual and buccal mucosal reaction. Niacinamide, given orally in 100 mg. daily doses for three days (E.G.), or concentrated B complex vitamins, given parenterally and orally for four days (R.L.), were administered while antibiotic treatment continued. All symptoms, except the bitter taste of the saliva, were relieved in both patients within 24 hours, and objective findings were markedly reduced in 48 hours. The clinical signs disappeared completely by the fourth to fifth day after vitamin administration was begun, although chloramphenicol therapy was continued.

Summary and conclusions

Two chronic typhoid carriers showed high, nearly constant levels of excretion of phage types E_1 or F_2 of *S. typhosa* during 14 weekly examinations of feces prior to treatment with chloramphenicol. The bile of both carriers, obtained by duodenal intubation, contained large numbers of typhoid bacilli on at least one occasion.

Chloramphenicol was administered in dosages of from 22 to 44 mg./kg./day for 15 or 19 days. The quantities of antibiotic in serum and urine were sufficient to suggest adequate absorption from the intestine, but little or no active chloramphenicol was detected in samples of bile obtained by duodenal intubation.

It is not certain whether high biliary excretion of chloramphenicol is required for successful treatment of the biliary typhoid carrier state. Woodward *et al.*,[10] in studies of concentration of chloramphenicol in bile, found levels approximately one-half as great as the levels detected in samples of serum obtained simultaneously. In patients with normally functioning gallbladders these levels should exceed the limit of sensitivity of the organisms carried by the subjects in this study. Both patients presented here showed evidence of gallbladder disease, so that if high concentrations of chloramphenicol in bile are necessary, these patients may have been at a disadvantage because of their reduced capacity to concentrate hepatic bile. It would seem, further, that if continued studies of the quantity of chloramphenicol in the bile are pertinent, it would be more profitable to obtain samples of bile directly from the biliary system rather than by duodenal intubation.

Only slight suppression of the fecal excretion of *S. typhosa* was noted during treatment with chloramphenicol, and the large quantities of typhoid bacilli observed originally were found in the feces immediately after cessation of therapy. This confirms the observations of other investigators who report little effect by chloramphenicol upon the typhoid carrier state.

An untoward oral reaction, resembling that caused by vitamin B deficiency, appeared in one patient within five days, and in the other within seven days after initiation of oral administration of chloramphenicol. Reduction of dosage for one patient did not influence the reaction. When niacinamide or a multiple B vitamin preparation was administered, prompt improvement of the oral reaction occurred while chloramphenicol administration was continued. Similar amelioration of these side effects of chloramphenicol have been reported by Harris.[6] Although neither patient had shown clinical evidence of vitamin B deficiency before treatment, the diet of one patient had been insufficient in vitamins of the B group, and the diet of the other had been marginal in this regard. It is possible that their dietary backgrounds contributed to the untoward reactions experienced by these patients.

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AN ANATOMICAL STUDY OF THE HUMAN SPINAL COLUMN

With Emphasis on Degenerative Changes in the Cervical Region*

DAVID EMERY MORTON

Since the work of Schmorl[21] in 1927, there has been growing interest in the study of the various degenerative changes associated with the aging process in the spinal column, especially in the intervertebral discs. The early developmental anatomy of the spinal column has received special attention in the attempt to explain some of these degenerative changes. This paper deals with a study of these changes in unselected cadavers.

The developmental anatomy of the vertebral column has been described by several investigators.[2, 4, 16, 20] The notochordal origin of the nucleus pulposus of the intervertebral disc, and the progressive decrease in its mucoid content during fetal life have been outlined by Keyes and Compere.[16] The nucleus pulposus is variously described as being large and situated somewhat posterior to the center of the disc,[10] or it is said to vary widely in size and position.[23] Progressive degenerative changes occur in the intervertebral disc throughout life.[16, 23] From the eighteenth to the seventieth year the water content of the nucleus pulposus falls from about 80 per cent to between 70 and 75 per cent.[19]

The relative avascularity of the intervertebral disc is an important factor in the production of degenerative changes, but the nature of the meager vascular supply is controversial.[8, 9, 11, 18, 24] Defects left in the cartilage plates of the vertebral bodies by atrophied pre-pubertal blood vessels may explain the formation of Schmorl's nodules.[9] Schmorl[21] found nuclear prolapses into the vertebral centra in 38 per cent of his routine autopsies, with a slightly higher incidence in males, and in the middle-age groups. Lumbar Schmorl's nodules have been reported in from 9.3 to 20 per cent of cadavers in various series.[7, 14]

A brownish discoloration termed "brown degeneration" has been described in the intervertebral discs, the etiology and nature of which are not definitely known.[15, 22] "Fissuring" is another change described in senile intervertebral discs.[15]

Posterior herniations of the nucleus pulposus through the annulus fibrosus are usually lateral to the posterior longitudinal ligament and may compress the spinal nerves against the vertebral pedicles or articular processes.[10] In the cervical region, however, herniations may lie close enough to the midline to produce compression of the spinal cord. Andrae[1] found posterior herniations in 15.2 per cent of 368 spinal columns, with the greatest number in the lower dorsal and lumbar regions. Lumbar herniations posteriorly have been described in 5.3 to 16 per cent of various series of cadavers,[7, 14] and anterior herniations in 6 per cent.[7] Horwitz[14] found fifty instances of bulging of the annular fibers without actual herniation of the nucleus pulposus. Artificial puncture of the annulus fibrosus has produced herniations in rabbits[12] and human cadavers.[5]

Horwitz[15] noted one instance of posterior herniation and three of ossification of the intervertebral disc in the cervical region of fifty cadavers; however, other degenerative

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changes were found in thirty-eight, especially in the lower cervical region. When the entire disc was thinned, he noted a widening of the intervertebral foramina, whereas a loss of only the posterior part of a disc caused narrowing of the foramina. Arthritic changes have been observed in some zygapophysial joints following nuclear herniations and thinning of the discs.[18]

Narrowing of the intervertebral discs is described after the formation of Schmorl's nodules and in the course of decalcifying bone diseases. Eburnation of the vertebral bodies and bony exostoses on their margins may develop after thinning of the discs.[10]

Clinical reports have indicated the possible production of neurological manifestations through compression of the spinal cord or nerves by hypertrophic arthritis of the posterior edges of the vertebral bodies.[3, 6, 17, 18]

Methods and materials

Spinal columns were obtained from forty cadavers. The laminae and ligamenta flava were cut parasagittally on each side of the spinous processes throughout the length of the spinal column. The spinous processes were then removed, exposing the contents of the spinal canal. The nerve roots were sectioned just medial to the intervertebral foramina, and the meninges and spinal cord were removed from the spinal canal and examined.

The spinal canal was inspected, and the remainder of the spinal column was divided into right and left halves by a midsagittal cut through the centra. The various structures in the cervical region were dissected and measured. The atlas was missing or destroyed in many of the specimens, and was not included in the series. Regions other than the cervical were not dissected completely unless special conditions warranted it. In each specimen, however, the fifth lumbar segment was disarticulated from the sacrum and closely studied.

The ages at death of thirty-six of the individuals from whom the specimens were taken were known. They ranged from 36 to 82 years, with an average of 64.6 years, and twenty-eight fell into the range between 55 and 75 years. Thirty-five spinal columns were from males, five from females.

Results—Conditions of the intervertebral discs

Brown degeneration and ossification. Brown degeneration was observed in one or more intervertebral discs in twenty-eight specimens (70 per cent) and was distributed throughout the spinal column (Table 1). Although it was impossible to distinguish a definite line of demarcation between the nucleus pulposus and the annulus fibrosus of the intervertebral discs, brown degeneration was most marked in the general area of the nucleus pulposus; however, in a few instances it involved the whole disc (Fig. 4). The affected area was reddish-brown in color, and was softer and more friable than the surrounding normal appearing tissue. The discs of both sexes were involved. The incidence was higher after 65 years, although the youngest specimen, aged 36 years, was also affected. Brown degeneration was not consistently associated with other spinal pathology.

Ossification was noted in an intervertebral disc in two instances. In one specimen the disc C6/7* was thin and its posterior portion partially ossified.

* In the interest of saving space, an abbreviated notation will be used for vertebrae, spinal nerves, intervertebral discs, and foramina, e. g., T2 indicates the second thoracic vertebra or nerves, and L2/3, the intervertebral disc or foramina between the second and third lumbar vertebrae.

In another specimen the vertebral bodies of C6 and C7 were fused, and the small remaining portion of the disc was ossified.

Fissuring. Crevices in the intervertebral discs longer than 3 mm. were observed in twelve specimens (30 per cent), and more frequently in the larger discs of the lower dorsal and lumbar regions, where normally the pulpy material in the area of the nucleus pulposus tended to protrude from the cut surface of the disc. In the upper dorsal and cervical regions where fissuring was observed infrequently, the central portion of incised discs did not tend to bulge, and it was difficult to detect a sharp line of transition between the nucleus pulposus and the annulus fibrosus (Table 1).

TABLE 1

THE DISTRIBUTION OF BROWN DEGENERATION OF THE INTERVERTEBRAL DISCS IN TWENTY-EIGHT CADAVERS, AND FISSURING IN TWELVE CADAVERS

	No. of specimens involved			*No. of specimens involved*	
Disc	*Brown degeneration*	*Fissuring*	*Disc*	*Brown degeneration*	*Fissuring*
C2/3	3	0	T7/8	4	0
3/4	6	1	8/9	6	0
4/5	3	1	9/10	4	0
5/6	5	0	10/11	3	4
6/7	6	0	11/12	4	4
7/T1	5	2	12/L1	4	3
T1/2	2	1	L1/2	2	2
2/3	3	0	2/3	4	1
3/4	4	1	3/4	1	2
4/5	3	0	4/5	2	2
5/6	5	1	5/S1	1	4
6/7	4	1			

Fissuring was noted most frequently in the central portion of the disc. Usually several fissures were present, and occasionally the whole disc was involved, even close to the cartilage plates. The fissures most commonly lay in a horizontal plane, and at times one communicated with another by a small opening. There was no correlation between fissuring and other changes of the intervertebral discs; however, it was more prominent in older specimens. Both sexes were involved.

Thinning. Measurements of the anterior, middle, and posterior thicknesses of the cervical intervertebral discs were made along the midsagittal plane (Table 2). Criteria were arbitrarily established to define thinning of the various portions of the discs in the midline, and the incidence in the different regions was noted (Table 3).

TABLE 2

THE AVERAGE THICKNESS OF THE CERVICAL INTERVERTEBRAL DISCS MEASURED IN THE MID-SAGITTAL PLANE

Disc	*No. of cadavers measured*	*Anterior edge of disc*	*Middle of disc*	*Posterior edge of disc*
C2/3	35	3.8 mm.	4.9 mm.	2.7 mm.
3/4	35	3.7	4.3	2.1
4/5	39	3.8	4.2	2.1
5/6	39	3.2	3.8	1.8
6/7	39	3.2	3.8	1.7
7/T1	40	3.5	4.5	2.4

TABLE 3

THE INCIDENCE OF THINNING OF THE INTERVERTEBRAL DISCS IN FORTY CADAVERS

Disc	*Number of specimens with thinning*		
	Uniform[1]	*Anterior only*[2]	*Posterior only*[3]
C2/3	1	3	0
3/4	6 In 18	2 In 10	9 In 22
4/5	6 specimens	2 specimens	4 specimens
5/6	10 (45 per	3 (25 per	11 (55 per
6/7	13 cent)	2 cent)	12 cent)
7/T1	3	3	0
Below: Thinning denoted only by markedly decreased thickness of disc as compared with normal adjoining discs. Accurate measurements not taken.			
T1/2	4	3	5
2/3	7	4	5
3/4	7	7	5
4/5	8	8	5
5/6	7 In 11	8 In 12	4 In 15
6/7	1 specimens	8 specimens	4 specimens
7/8	2 (27.5 per	5 (30 per	5 (37.5 per
8/9	1 cent)	5 cent)	5 cent)
9/10	4	4	5
10/11	1	4	6
11/12	1	5	10
12/L1	2	4	9
L1/2	1	1	10
2/3	0 In 4	1 In 4	10 In 27
3/4	0 specimens	1 specimens	12 specimens
4/5	0 (10 per	0 (10 per	13 (67.5 per
5/S1	3 cent)	1 cent)	21 cent)

[1] Equal to or less than: 2 mm. ant.; 3 mm. mid.; 1.5 mm. post.
[2] Equal to or less than 2 mm. ant.
[3] Equal to or less than 1.5 mm. post.

All the normal cervical discs were biconvex in shape in the median plane and were somewhat more than 1 mm. thicker anteriorly than posteriorly (Figs. 4, 6). The thickness ranged from 0 to 6 mm. anteriorly, 0 to 6 mm. through the center, and 0 to 5 mm. posteriorly. The discs of the older specimens and females were slightly thinner than those of the younger specimens and males.

Posterior thinning of the lumbosacral disc was observed in twenty-one specimens (52.5 per cent), and the fibers of the annulus fibrosus were completely absent posteriorly in two specimens.

The exact thickness of the cervical discs was not determined in planes other than the midsagittal, but by gross observation, no marked discrepancies were noted between the thickness of the right and left sides of any of the cervical discs. In two specimens, however, several cervical discs were absent or very thin on each side, where bony processes extended

TABLE 4

THE DISTRIBUTION OF SCHMORL'S NODULES BY AGE GROUPS IN THIRTY-ONE MALE SPECIMENS

Age groups	*No. of specimens*	*No. of specimens with Schmorl's nodules*	*Per cent of specimens involved*
30-39 years	1	1	(100.0%)
40-49	0	0	.....
50-59	7	3	42.9
60-69	13	7	53.8
70-79	10	7	70.0
80-89	0	0	.....

superiorly from the superolateral aspects of a vertebral body to enclose the inferolateral aspects of the centrum above. In one instance an actual joint space and synovial membrane were present (cf. Gray[12]).

Schmorl's nodules. Four of the twenty-seven specimens with intraspongious herniations of the nucleus pulposus were females (Fig. 4; Fig. 7; Table 4). In half the specimens with involvement between vertebrae T5 and L1 the nodules were observed in series, two to four consecutive vertebrae having been affected. In the majority of these series all the nodules passed cephalad, or all passed caudad from their respective discs in a given specimen. Of the forty specimens, 22.4, 42.5, and 10.0 per cent had Schmorl's nodules in the cervical, dorsal, and lumbar regions respectively. There was no correlation of the incidence of nodules in the three regions of a particular specimen.

In twenty-three specimens which showed thinned cervical discs, but no nodules in the midsagittal plane nor posterior herniations of the nucleus

pulposus, parasagittal sections were made at intervals of about 2 mm. Parasagittal nodules were detected in only two instances, as compared with eleven lying in the midline. In these two cases, the nodules lay about 3 mm. to the right of the midline and invaded the centrum of C7 from the disc above.

In all but three instances the defects in the cartilage plates through which the nuclear material prolapsed measured less than 1 mm. across. In one

TABLE 5

THE POSITION OF FIFTY-FOUR SCHMORL'S NODULES IN THE MEDIAN PLANE, AND THEIR INCIDENCE WITH AND WITHOUT THINNING OF THE DISCS

Disc	*Anterior to center of centrum*	*Center of centrum*	*Posterior to center of centrum*	*Nodules with thinning*	*Nodules without thinning*
Totals	3	10	41	15	39
C2/3		1		1	
3/4			2		2
4/5		1			1
5/6		4		3	1
6/7			1	1	
7/T1			1		1
T1/2-3/4	..	..	..	..	..
4/5		1			1
5/6			1	1	
6/7	1		4		5
7/8	1		8	5	4
8/9			6		6
9/10			5	1	4
10/11		1	1		2
11/12	1	1	4	1	5
12/L1		1	2	1	2
L1/2			1	1	
2/3			3		3
3/4			1		1
4/5			1		1
5/S1	..	..	..	..	..

instance much of both cartilage plates had been widely split and destroyed and there were massive prolapses of material from the discs above and below into the centrum of T12. The cartilage plates surrounding the defects were between 0.5 mm. and 1 mm. thick; however, bending of the plates into the vertebral centra close to the defects occurred frequently. Pronounced bending of the plates without the actual formation of Schmorl's nodules was also observed often, especially in the lumbar region.

In the cervical region the nodules measured 2 to 3 mm. in their vertical diameter, and 3 mm. in their anteroposterior and transverse diameters. In

the dorsal and lumbar regions, there was more variation in size, ranging from 1 to 9 mm. in diameter. The nodules were either oval or irregular in shape.

In sections of five nodules examined microscopically, the defect in the hyaline cartilage plate could be easily seen. Fibrocartilaginous material from the disc extended through the defect into the vertebral centrum. The nuclear area of the disc near the prolapse showed cracks and gaps in three instances, presumably occupied formerly by the prolapsed material. The formation of bone around the nodules was not noted in any specimen.

Forty-one of fifty-four nodules lay 2 to 3 mm. posterior to the middle of the vertebral body (Table 5). Of the three nodules which lay anterior to the center of the centra, the defect in the cartilage plate always lay at the center of the centrum, and the nodules themselves were not more than 2 mm. anterior to that point.

There was no correlation between the incidence of Schmorl's nodules and brown degeneration, fissuring, and eburnation. Lipping of the edges of the vertebral centra was an accompanying factor ten times. Fourteen of the twenty-seven specimens with Schmorl's nodules showed thinning of one or more discs involved in nodule formation (51.8 per cent), whereas seventeen specimens had one or more discs with nodules, but without thinning (63 per cent; Table 5).

Anterior and posterior herniations of the nucleus pulposus. Anterior herniation of the nucleus pulposus was noted only once, involving a disc L2/3. The herniation was incomplete, having pushed through all but the outermost layers of the annulus fibrosus.

Fourteen posterior herniations of the nucleus pulposus through the annulus fibrosus were noted in eight specimens (20 per cent; Fig. 7). All but two of the eight herniations which indented the cord lay almost directly in the midline, the herniating material having pushed partially or completely through the posterior longitudinal ligament. The other two herniations, one in the cervical and one in the dorsal region, indented the left side of the cord after having pushed through the lateral portion of the posterior longitudinal ligament. In the one posterolateral herniation between vertebrae C6 and C7, the disc was uniformly thinned to 1 mm., and the left nerve C7 was flattened and tightly wedged against the left superior articular process of vertebra C7 by the herniation and posterior lipping of the body of C7.

In one specimen there was an incomplete herniation of the lumbosacral disc which slightly compressed the left nerve L5. A similar condition existed in another specimen between T12 and L1, but without nerve compression.

The herniations appeared as regular, moderately firm, whitish knobs, which measured about 3 mm. across, and elevated or pushed through the

posterior longitudinal ligament, extending 2 to 4 mm. into the spinal canal. On gross inspection of transverse sections, such discs showed the rupture of the annulus fibrosus, and the path of the nuclear material through it. The midline herniations thinned and indented the anterior portion of the dura mater. The largest cervical and dorsal herniations indented the spinal cord from 1 to 3 mm., either extending into both ventral white columns and compressing the anterior spinal artery, or extending to one side slightly to press on some of the ventral nerve roots. There was no gross or micro-

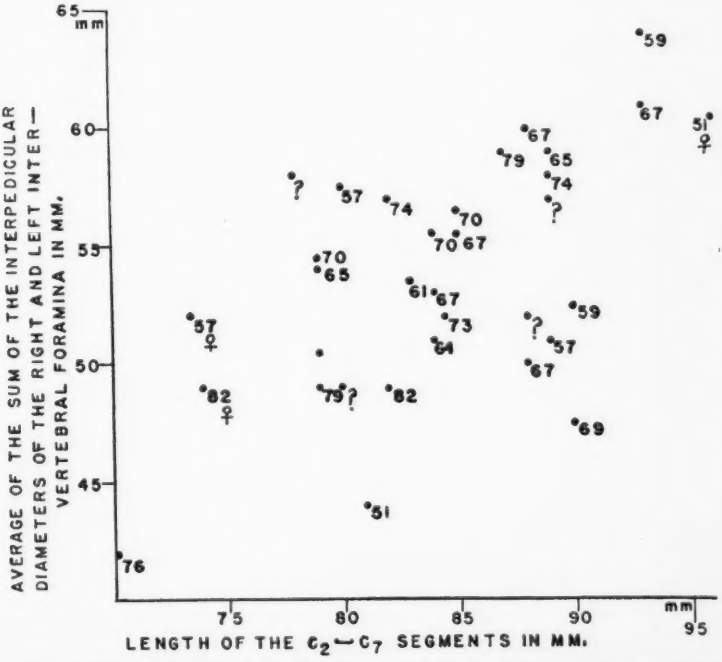


FIG. 1. The relationship of the C2-C7 segments to the average of the sum of the right and left interpedicular diameters of the intervertebral foramina is indicated in thirty-three cadavers. The ages are marked, where known, as are the female specimens.

scopic evidence of degeneration of the cord in these specimens, although the anterior spinal artery and its adjacent branches were flattened so that the lumina were mere slits.

The ages of the specimens with posterior herniations varied from 52 to 82 years. Three specimens were females. Brown degeneration and fissuring of the discs, and eburnation and lipping of the adjacent vertebral bodies were not regularly associated with posterior herniations. Two cervical discs with posterior herniations were thinned posteriorly, and two were uni-

formly thinned. Two of the thoracic herniations were accompanied by uniform thinning; seven were not. The one instance at the lumbosacral disc was accompanied by marked posterior thinning, and there was also posterior lipping of the bodies of L5 and S1.

Posterior protrusions of the fibers of the annulus fibrosus. By this condition is meant a projection of the posterior annular fibers into the vertebral canal, which pushes the posterior longitudinal ligament posteriorly at that point. Only those fibers which protruded more than 2 mm. into the

TABLE 6

THE INCIDENCE OF POSTERIOR PROTRUSIONS OF THE ANNULUS FIBROSUS AND ASSOCIATED THINNING OF THE DISCS IN THIRTY-ONE CADAVERS

Discs	*No. of specimens with protrusions*	*Indentation of cord*	*Nerve or nerve root compression*	*Uniform thinning of disc*	*Anterior thinning of disc*	*Posterior thinning of disc*
C2/3	4					1
3/4	5	2				2
4/5	6	2		1		2
5/6	6	2	1	1		5
6/7	5	1		1		2
7/T1	1				1	
T1/2-7/8	..	..	..	..	..	..
8/9	2			1		1
9/10	1					1
10/11	2	1	1			1
11/12	5	2	1			3
12/L1	6	1	1			3
L1/2	5					4
2/3	5			3		1
3/4	5					2
4/5	14					10
5/S1	27		3			20
L5/Transitional vertebra	1		1, NS1			
Totals	100 protrusions in 31 specimens	11	8	7	1	58

vertebral canal were classified in this category. Incomplete herniation of the nucleus pulposus was not included.

Thirty-one of the forty specimens (77.5 per cent) showed involvement of one or more intervertebral discs (Table 6). The relative incidence and degree of nerve and spinal cord compression produced by annular protrusion were less than those associated with nuclear herniation, which occurred

infrequently but often caused nerve or cord involvement. The nerve L5 was compressed slightly only three times in the twenty-seven lumbosacral annular protrusions.

Grossly the condition resembled posterior herniations of the nucleus; however, the annular fibers extended less into the vertebral canal, and the whole width of the disc was involved instead of the localized area noted in nuclear herniations. Posterior annular protrusion was frequently found in specimens over 50 years of age in the discs L3/4 and L4/5, and somewhat less frequently in the dorsal and cervical regions. Four of the five female specimens were involved. Thinning of the discs posteriorly was the only

TABLE 7

THE AVERAGE MEASUREMENTS OF THE CERVICAL VERTEBRAL CENTRA IN THE MEDIAN PLANE

A. Craniocaudal measurements

Vertebral centrum	*No. of specimens measured*	*Anterior*	*Middle*	*Posterior*
C2	..	...	...	...
C3	38	13.9 mm.	11.0 mm.	13.6 mm.
C4	39	13.7	11.9	13.5
C5	39	12.4	11.1	13.5
C6	39	12.4	11.4	13.1
C7	40	14.1	13.0	14.5
T1	40	15.9	15.4	16.9

B. Dorsoventral measurements

Vertebral centrum	*No. of specimens measured*	*Superior*	*Middle*	*Inferior*
C2	34	...	...	16.1 mm.
C3	38	15.3 mm.	14.8 mm.	16.5
C4	39	16.9	15.1	17.4
C5	39	17.1	15.9	18.5
C6	39	17.8	16.1	18.8
C7	40	18.7	16.4	17.8
T1	40	17.9	17.2	18.7

feature accompanying posterior annular protrusions with any regularity (Table 6). Schmorl's nodules were present in three of the dorsal discs with annular protrusions.

Conditions of the vertebral bodies

Measurements of the cervical and first thoracic vertebrae. The height of each centrum from C3 to T1 was measured at its anterior and posterior edges and through its center in the midsagittal plane, and the antero-

posterior dimensions were determined at the superior and inferior edges and through the center (Table 7). Transverse dimensions, and the heights of the centra in parasagittal planes were not determined. Only the inferior edge of the axis was measured.

There was considerable variation in the size of the vertebrae in different specimens, and no constant relationship existed between the heights and anteroposterior dimensions of the vertebral centra; however, in twelve specimens the decreased heights of the bodies of C5 and C6 were accompanied by an increase in the width and depth of the same centra, with lateral bony lipping, as though the bodies had been flattened out. The anteroposterior measurements were slightly greater along the superior and inferior borders than through the middle of the vertebral bodies. This condition was exaggerated where there was hypertrophic bony lipping of the vertebral centra. The centrum of T1 was more nearly square midsagittally than the cervical vertebral bodies, which were rectangular.

TABLE 8

THE INCIDENCE OF EBURNATION OF THE ADJOINING EDGES OF THE VERTEBRAL BODIES IN NINE CADAVERS

Vertebral bodies	*Number of specimens involved*
C2&3	1
3&4	2
4&5	5
5&6	5
6&7	3
7&T1	1
T1&2	1
2&3	1
3-9	0
9&10	1
T10-L2	0
L2&3	1
L3-S1	0

Eburnation. Eburnation, or the conversion of bone into an ivory-like mass, occurred more frequently at the edges of the vertebral bodies adjoining markedly thinned intervertebral discs. The process usually did not extend more than 3 mm. into the spongiosa of the bodies, but when there was marked destruction of the intervertebral disc, half or more of the adjacent bodies were involved. Eburnation was more advanced in the region of the centra nearest the thinnest part of the disc, which was most frequently the anterior or posterior edge. Both vertebrae adjoining a disc were affected in all but one instance (Table 8).

Grossly the eburnated areas resembled ivory. They were lighter in color and harder than normal cancellous bone, and the cut surface was shiny

(Fig. 3). Microscopically the eburnated areas were seen to begin where the thin layer of cortical bone abutted on the cartilage plate. Thick trabeculations were arranged in a much denser network than in the normal spongiosa. Many of the marrow spaces were partly obliterated. Farther in the centrum the eburnated areas gradually merged with the normal cancellous bone. The cartilage plates were intact in most instances, but occasionally they were cracked and less than 0.5 mm. thick.

The intervening intervertebral discs were markedly thinned in 85 per cent of the instances of eburnation, and to a lesser degree in the remaining 15 per cent. Eburnation was not closely correlated with other changes involving the discs or centra. It was not seen in the five female specimens. The ages of seven of the nine specimens were known, and with the exception of one aged 51 years at death, all were 67 years or older.

TABLE 9

THE INCIDENCE OF ANTERIOR AND POSTERIOR LIPPING OF THE ADJACENT EDGES OF THE VARIOUS VERTEBRAL BODIES IN FORTY CADAVERS

Position of vertebrae	*No. of specimens with lipping*		*Position of vertebrae*	*No. of specimens with lipping*	
	Anterior	*Posterior*		*Anterior*	*Posterior*
C2&3	8	5	T7&8	9	2
3&4	17	16	8&9	10	2
4&5	21	21	9&10	9	2
5&6	26	26	10&11	8	3
6&7	26	25	11&12	11	4
7&T1	17	12	12&L1	17	5
T1&2	9	5	L1&2	17	6
2&3	10	1	2&3	16	7
3&4	13	1	3&4	14	4
4&5	10	1	4&5	15	6
5&6	11	1	5&S1	7	6
6&7	10	2			

Lipping. Hypertrophic lipping was most prominent over the anterolateral edges of the vertebral bodies. These exostoses closely resembled the eburnated areas. Lipping of one or more vertebral bodies along the anterior or anterolateral edges was noted in thirty-six specimens (90 per cent). The exostoses projected from 2 mm. to 15 mm. from the vertebral bodies; they either involved one or more localized points, or extended completely around the circumference of the anterior half of the centrum, and in two specimens completely bridged several discs (Figs. 3, 6).

Posterior lipping into the vertebral canal was noted on one or more vertebral bodies of thirty-two specimens (80 per cent). The bony processes extended from 1.5 to 4 mm. into the vertebral canal (Fig. 6). Although lipping of the posterior edges of two adjacent vertebrae usually extended

across the whole width of the vertebral canal, it was often more marked on one side (Fig. 5, Table 9). If posterior lipping of one vertebral body was present, it would also be present on the adjacent edge of the vertebra on the other side of the disc. Anterior lipping was more frequent and greater in extent in all but the cervical region.

No constant correlation was found between lipping and changes in the intervertebral discs. One of the fourteen instances of posterior herniation of the nucleus pulposus showed lipping at a corresponding level (C6 and C7). Thirteen of the twenty-seven specimens with Schmorl's nodules had lipping of the adjacent vertebral bodies (at fourteen disc levels as compared with the total of sixty-two discs with Schmorl's nodules). Forty per cent of the levels showing anterior lipping were accompanied by anterior thinning of the intervertebral discs, whereas 50 per cent of the levels with posterior lipping showed an accompanying posterior thinning throughout all regions.

Lipping was noted in all five female specimens, and in 88 per cent of the thirty-five males. It was most prevalent in specimens from the eighth and ninth decades.

Posterior lipping compressed, or made impressions in, the spinal cord in nine specimens, with the highest incidence in the cervical region (Fig. 7). The lipping pushed the posterior longitudinal ligament and dura mater into the ventral surface of the spinal cord to a depth of about 1 mm., except in one specimen where the lipping was very marked (Fig. 2; Fig. 6). Microscopically the latter specimen was unsatisfactory because of the dryness and friability of the tissues; however, the anterior spinal vessels were flattened and only thin slits remained for lumina.

Compression of spinal nerves by encroachment of posterolateral exostoses on the intervertebral foramina occurred in eight specimens (Fig. 7). The most significant observations were limited to the cervical region. In seven instances nerves were compressed on the left side, and in three the right nerves were involved.

The nerves were invariably pressed against the superior articular process or lamina of the vertebra forming the inferior half of the circumference of the intervertebral foramen by the hypertrophied edges of the vertebral bodies (Figs. 5, 6). In one specimen marked hypertrophic arthritic changes of the articular process contributed to the compression, along with posterolateral lipping (Fig. 6). In none of the other nine instances were definite hypertrophic changes of the articular processes observed; however, in three the superior articular process was larger than normal, its anterior aspect projected unusually far into the intervertebral foramen and thus contributed to the latter's decreased anteroposterior diameter.

The involved nerve was in each case flattened in proportion to its degree of compression, and clamped firmly in position. Two nerves studied micro-

scopically showed no changes; however, a third nerve manifested marked venous engorgement proximal and distal to the point of compression, and poorly stained nerve fibers interspersed with a yellowish pigment (hematoxylin and eosin stain) in the compressed region. Grossly this specimen, a left nerve C6, appeared swollen distal to the point of compression and showed a bluish discoloration.

Conditions of the intervertebral foramina

Measurements. The interpedicular and anteroposterior diameters of the cervical intervertebral foramina were measured with a ruler. The latter diameters actually faced 45 degrees anteriorly, and were measured between the intervertebral disc anteriorly and the anterior edge of the superior articular process posteriorly (Table 10). The cervical intervertebral foramina resembled a figure eight with its transverse bar missing. The interpedicular diameters varied between 6.5 mm. and 13.1 mm.; however, the majority of them were within 1 mm. of the average listed. Variations in the anteroposterior diameters ranged from 0 mm. to 8 mm., with wide variations from the averages, and did not exceed 3 mm. in any case of nerve compression.

TABLE 10

THE AVERAGE MEASUREMENTS OF THE CERVICAL INTERVERTEBRAL FORAMINA

Foramina	*No. of specimens measured*	*Interpedicular diameter*		*Anteroposterior diameter*	
		Right	*Left*	*Right*	*Left*
C2/3	33	10.1 mm.	10.0 mm.	6.1 mm.	5.4 mm.
3/4	34	8.4	8.1	4.4	4.3
4/5	38	8.2	8.4	4.1	4.1
5/6	38	9.0	8.7	4.3	4.3
6/7	38	8.8	8.6	4.8	4.8
7/T1	39	9.4	9.1	5.7	5.6

The foramina were slightly smaller in the female specimens than in the males, and those of the larger specimens were correspondingly large in size. This was shown by comparing the average of the sum of the interpedicular foraminal diameters of each specimen with the distance between the inferior borders of the centra of C2 and C7 (Fig. 1).

Changes associated with decrease in size of the intervertebral foramina. Foramina with interpedicular diameters less than 7.5 mm. and anteroposterior diameters less than 3.5 mm. were arbitrarily compared with the incidence of other changes in the discs and vertebrae. Of the specimens with interpedicular foraminal diameters less than 7.5 mm. (fourteen foramina), 83.3 per cent had uniform or posterior thinning of the cor-

responding intervertebral discs. Seventy-five per cent of the specimens with an anteroposterior foraminal diameter less than 3.5 mm. (sixty foramina) also showed uniform or posterior thinning of the discs.

Studies on disarticulated cervical vertebrae from which the discs had been removed indicated that when the vertebral bodies were moved closer together, as would happen in uniform thinning of the discs, the interpedicular diameters of the corresponding foramina decreased proportionately, as did the anteroposterior diameters to a lesser extent.

Seventy per cent of the specimens with anteroposterior foraminal diameters less than 3.5 mm. showed posterior lipping of the corresponding vertebral bodies, the remaining 30 per cent, annular protrusions or nuclear herniations (Fig. 5). Of the fourteen foramina with small interpedicular diameters, 87.5 per cent had corresponding posterior lipping.

Conditions of the zygapophysial joints

Arthritic changes. In the cervical region, arthritic changes in the zygapophysial articulations were observed in six specimens (15 per cent). The incidence was slightly higher on the left side. Seventy per cent of the arthritic changes were hypertrophic in nature; in 30 per cent they were atrophic. In the latter there were frequently additional hypertrophic changes around the margins of the articular processes, suggesting a combination of hypertrophic and atrophic arthritis (Fig. 6). In one instance of marked lipping of the vertebral bodies, one zygapophysial articulation was ankylosed by lipping of its edges (Fig. 3). The lipping was minimal in the other articulations, but there was a moderate amount of pitting and erosion of the cartilage plates. Most of the hypertrophic articular changes were noted in specimens over 65 years of age.

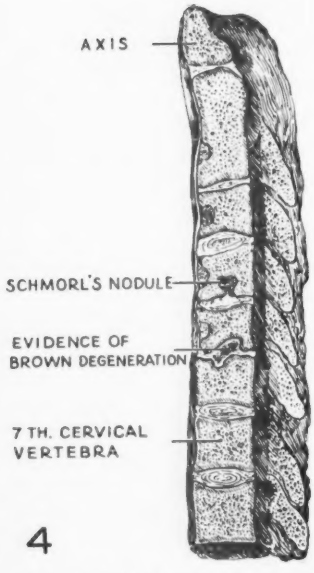
In nine of the twenty-six articulations involved in arthritic changes, the anteroposterior diameters of the corresponding intervertebral foramina were noticeably decreased; however, articular arthritis obviously contributed to nerve compression in only one instance, posterior vertebral lipping having been the chief factor. Schmorl's nodules were noted at corresponding levels three times. In each case of arthritis there was thinning of the disc, and in one instance the latter was almost completely obliterated.

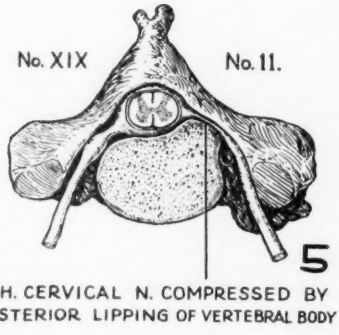
Unusual and anomalous conditions

An extra transitional vertebra was interposed between the "fifth" lumbar vertebra and the sacrum in one male and one female specimen (aged 67 and 82 years). The vertebral columns and their contents were otherwise normal, and a nerve passed through each intervertebral and sacral foramen; however, the first cervical and coccygeal nerves were not found.

There was almost complete fusion of the centra of C6 and C7 of one 79-year-old male. The only trace of the intervertebral disc was a thin layer of calcified material.

FIG. 2. The ventral portion of the dura mater has been reflected inferiorly, exposing the ventral surface of the spinal cord in the cervical region. The indentation of the cord and dura was caused by posterior lipping of the cervical vertebral bodies (cf. Fig. 6). x1. (Courtesy of Am. J. Roentg.)





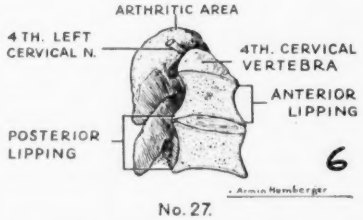


FIG. 3. Sagittal section showing the left half of the cervical region. Marked thinning of the discs and vertebral eburnation and lipping, especially anteriorly where some of the discs are bridged by bone, are shown. x2/3.

FIG. 4. Sagittal section showing the right half of the cervical region. Anterior lipping, disc thinning, a Schmorl's nodule, and brown degeneration are illustrated. The cervical curvature has been almost completely obliterated. x2/3. (Courtesy Am. J. Roentg.)

FIG. 5. Semidiagrammatic drawing showing the superior portion of vertebra C7 and compression of the left nerve C7 by posterolateral lipping of the vertebral body. x2/3. (Courtesy Am. J. Roentg.)

FIG. 6. Sagittal section showing the left half of vertebrae C4 and C5. Marked anterior and posterior lipping are present. The latter indented the cord as shown in Fig. 2. Compression of the left nerve C4 by posterior lipping and articular arthritis is also present. x2/3. (Courtesy of Am. J. Roentg.)

In one cadaver the laminae of C3 and C4 were fused, and the zygapophysial joints were completely ankylosed bilaterally.

One 65-year-old male specimen revealed a dilatation of the sacral canal at the level of S2, measuring 22 x 18 x 20 mm. The cavity contained a collapsed, cystic structure without signs of inflammation.

There was one instance of dislocation of the left zygapophysial joint C6/7, in which the spinal cord and nerve had not been compressed.

Spondylolisthesis was not observed in any case.

Discussion

It is likely that brown degeneration is not due to degenerating blood pigments unless one assumes that the normally avascular disc has become vascularized, or that blood has seeped into the discs from damaged vertebral arteries or veins. A more likely theory would be that the discoloration is the result of changes in the tissues of the disc.

The term fissuring was not applied unless the spaces within the discs were at least 3 mm. long, a purely arbitrary value; however, smaller defects were found in almost every disc. Some of the fissures may have been artefacts, but many were also found when the discs were carefully incised with a sharp scalpel.

The criteria for thinning of the cervical discs differed from those in other regions, and comparisons between the cervical and other regions should not be interpreted too strictly.

Considering that there are twice as many discs in the dorsal as in the cervical region, the probability of involvement of one disc in the formation of a Schmorl's nodule in either region would be about the same. The lumbar region, however, had the smallest ratio of nodules to the total number of discs, indicating both a relative and absolute minority in this region.

The integrity of the cartilage plates seems to be important in the prevention of the formation of these nodules. Three mechanisms are postulated as leading to a prolapse through the weakest part of the cartilage plate, perhaps where the notochord or vessels passed through the plates originally: (1) trauma; (2) defects in the compact or the adjacent cancellous bone, either due to osteoporosis or congenital weakness; (3) a combination of the above. The increased incidence of nodules with advancing age may indicate that factors other than congenital ones, such as trauma and senile degeneration, are important, although congenital weak points may be primary factors. The latter idea is reinforced by the observation that the cervical nodules almost always lay in the median plane (although a few in parasagittal positions may have gone undetected), slightly posterior to the center of the centra, in a position corresponding to that of the notochord in early development.

It is seriously questioned from these studies whether Schmorl's nodules are prime factors in producing degenerative changes in the intervertebral

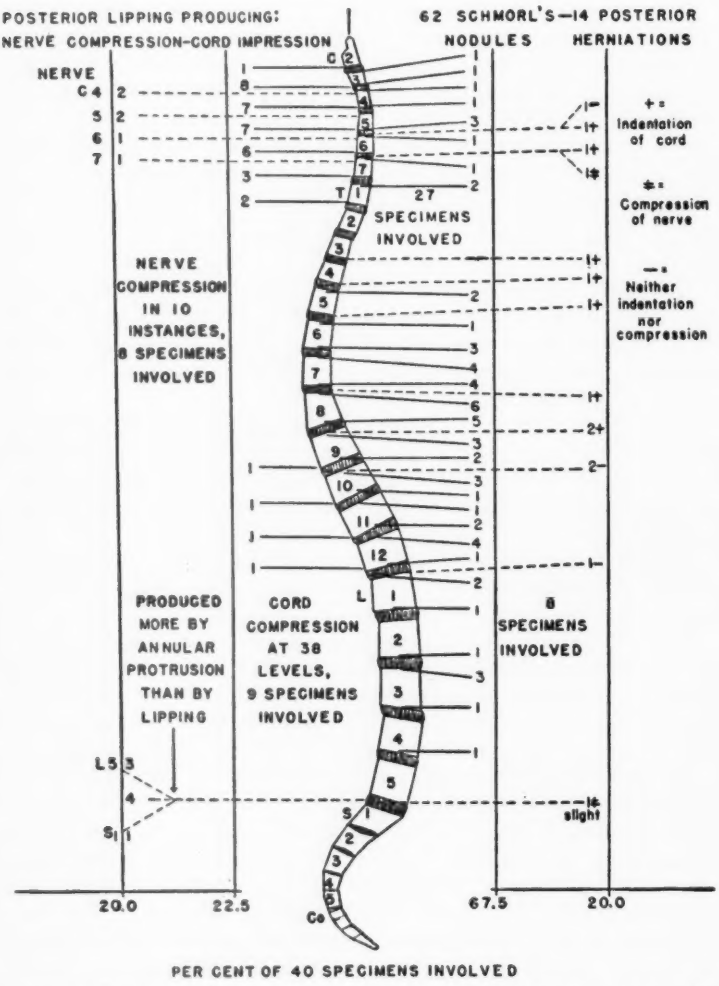


FIG. 7. The incidence of various conditions at different levels in forty spinal columns is indicated.

discs and their sequelae. Many discs appeared otherwise completely normal, and many discs showed advanced degenerative changes without Schmorl's nodules. There was no correlation between the size of the nodules and degenerative changes in the discs, except in the instance of the few large nodules measuring more than 5 mm. in diameter.

It is not known if the posterior herniations caused symptoms before the death of the individuals. The herniations may have occurred slowly, in which condition the collateral circulation in the cord could probably have kept up with the gradually increasing pressure. On the other hand, if they had occurred abruptly, symptoms probably would have been produced, and more microscopic evidence of damage to the cord would have been evident.

The frequent involvement of the lumbosacral disc in posterior annular protrusions may be attributed to the large forces it must withstand, and the transition between the movable lumbar region and fixed sacrum. Posterior annular protrusion to a slight extent is normal in the extended spinal column, and the inclusion of discs with the extension of annular fibers more than 2 mm. posteriorly is purely arbitrary. Trauma and ageing are probably responsible for pathological degrees of this condition.

The higher incidence of eburnation in the lower cervical region, and the diminished heights of the bodies of C5 and C6 may also have been the result of increased wear associated with the greater mobility of the segments above the thoracic region.

Although eburnation of the vertebral bodies was always associated with thinning of the intervertebral discs, the latter must have been marked and of fairly long duration, if it was the cause of eburnation at all, since the majority of moderately thinned discs were not accompanied by this condition.

In some of the specimens it was difficult to determine whether lipping resulted from hypertrophy of the edges of the vertebral bodies, or bony resorption from the middle of the anterior and lateral surfaces of the centra, leaving the edges of the latter protruding prominently. In the latter instance lipping would have been the result of a different mechanism from hypertrophic lipping. Neurological symptoms may actually occur infrequently in cases of posterior lipping because of adaptation of the nervous system and its blood supply to the slowly increasing pressure.

Narrowing of the anteroposterior diameters of the intervertebral foramina did not prove to be so significant as narrowing of the foramina in their inferior halves, where nerve compression actually took place. It is difficult to explain the large size of foramina C2/3, but the gradual increase in size of foramina C3/4 to C7/T1 would be in keeping with the increasing size of the nerves C4 to C8.

Summary

1. The spinal columns of forty cadavers were dissected, with attention concentrated on the cervical region.

2. Brown degeneration of the intervertebral discs was observed in twenty-eight specimens (70 per cent) and was not closely correlated with other degenerative changes.

3. Fissuring of the intervertebral discs was observed in 30 per cent of the specimens.

4. The thicknesses of the cervical discs were measured in the mid-sagittal plane. Posterior thinning was observed more frequently in the cervical and lumbar regions, accentuating the normal cervical and lumbar curvatures, whereas anterior thinning of the discs was more prominent in the thoracic region.

5. Schmorl's nodules were noted in twenty-seven cadavers (67.5 per cent), with a higher incidence in older specimens. There was no constant correlation between Schmorl's nodules and other forms of spinal pathology. It is questionable whether Schmorl's nodules are prime factors in producing degenerative changes in the intervertebral discs unless they are quite large.

6. A partial anterior herniation of the nucleus pulposus through the annulus fibrosus was noted once. Fourteen posterior herniations were observed in eight specimens (20 per cent). Eight herniations indented the spinal cord, and one aided in compressing a left nerve C7. All but two herniations lay in the midline. The dorsal, cervical, and lumbar regions were involved in that order of frequency. Degenerative changes of the discs and vertebral bodies were not consistently associated with herniations. Thinning of the involved discs was not specifically characteristic of posterior herniations.

7. Posterior protrusions of one or more annulus fibrosus 2 mm. or more into the vertebral canal occurred in 77.5 per cent of the specimens, with the highest incidence at the lumbosacral disc and in the more movable cervical and lumbar regions. Cord and nerve compression were noted eleven and seven times respectively. Posterior thinning of the discs was the only constantly accompanying feature.

8. The vertebral centra from C3 to T1 were measured in the mid-sagittal plane. Vertebrae C5 and C6 were usually shorter than the others, but frequently wider, with some lipping, and gave the impression of having been flattened by pressure or wear.

9. Eburnation of the vertebral bodies was noted at twenty-one disc levels in nine cadavers, predominantly in the cervical region. Thinning of the corresponding discs consistently accompanied eburnation, suggesting that altered physiology resulting from advanced thinning and degeneration was the cause of the condition. It did not accompany other degenerative changes with any consistency.

10. Anterolateral and posterior hypertrophic lipping of the edges of the vertebral bodies was noted in 90 and 80 per cent of the specimens respectively. Posterior lipping was less extensive. Lipping did not consistently accompany other changes; approximately half the discs were normal. It is questionable whether lipping is secondary to thinning of the discs. Posterior lipping at thirty-eight levels in nine specimens (22.5 per cent) caused

indentation of the spinal cord and occurred most frequently in the cervical region. Ten spinal nerves were compressed in eight specimens (20 per cent) by posterolateral exostoses that were noted mainly in the cervical region. The nerves were compressed against the superior articular process of the corresponding vertebrae in the inferior half of the foramen. Posterior lipping may thus produce neurological manifestations, and must be differentiated from other conditions.

11. The interpedicular and anteroposterior diameters of the intervertebral foramina were measured. Posterior lipping was the predominant cause of decrease in the latter diameters. Uniform thinning of the cervical discs appeared to produce a decrease in the interpedicular diameters.

12. Hypertrophic or mixed arthritis of the articular processes was observed in six specimens (15 per cent).

13. Various unusual and anomalous conditions were noted.

ACKNOWLEDGMENT

Professor W. U. Gardner's interest and help have been major factors in the completion of this project. Mr. Armin Hemberger has kindly done the drawings.

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NEWS AND COMMENT

On Yale Research Reported in Other Journals

Immunology of the Coxsackie viruses. A summary of present knowledge of the Coxsackie group of viruses, which has just appeared in *Federation Proceedings,** includes an account by Melnick and Kraft of their studies on the differentiation of immunological types of these viruses. Their study represents one phase of a research project carried on for the past two years by Drs. Edward C. Curnen, Ernest W. Shaw, Joseph L. Melnick, and their associates in the Section of Preventive Medicine. First isolated by Dalldorf and Sickles from feces of two boys suffering from a paralytic illness, the virus was named after their village, Coxsackie, New York, located about twenty-five miles south of Albany on the Hudson River. Laboratory study has revealed the virus to differ immunologically from poliomyelitis virus, and to cause no signs or lesions of the latter disease when injected into monkeys. Coxsackie virus is, however, pathogenic for infant mice and hamsters, and it is now customary to include in one group all filtrable agents of small particle size showing this unique pathogenicity, although it has become clear that such agents are in some respects rather heterogeneous. Dalldorf* classifies them as *group A,* inducing lesions only in skeletal muscles (of immature mice and hamsters), and *group B,* producing lesions in the brain, heart and other organs.

Clinical studies suggest that Coxsackie viruses may cause a variety of symptoms in man, including fever, malaise, headache, sore throat, backache, pain in the chest or abdomen, nausea, vomiting, ocular myalgia, and stiffness of the neck or back.† In different patients the illness may have what appear to be quite different manifestations, simulating in one case poliomyelitis, in another, pleurodynia, sinusitis, influenza, or appendicitis. Its relation to poliomyelitis is not fully understood, although Coxsackie virus has been isolated, sometimes alone, sometimes along with poliomyelitis virus, from the feces of patients with "polio." These viruses appear to be widely distributed, since they have been recovered from pharyngeal swabbings and from feces, as well as from sewage and from flies, in many scattered localities in this country, while antibodies have been discovered in human blood collected in Denmark, Sweden, and Alaska.

An interesting section of Melnick and Kraft's paper describes an epidemiological study done within the Melnick family, where two distinct strains of the virus were identified as having been present at about the same time. One of them, known as Conn.-5, had already been under investigation; the other, now designated by the name of Dr. Melnick's daughter, Nancy, then eight years of age, was subsequently found to differ from all of the other strains tested. By submitting the various strains available to tests of cross neutralization, cross complement fixation, cross protection in infant mice born of vaccinated mothers, and cross protection in chimpanzees with subclinical infections, it was possible to isolate seven strains which appeared to be distinct types.

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* American Association of Immunologists: Symposium on Coxsackie group of viruses. Fed. Proc., Balt., 1950, *9*, 569, 570, 574, 581, and 585.

† Curnen, E. C.: Human disease associated with the Coxsackie viruses. Bull. N. York Acad. M., 1950, *26*, 335.

Pulmonary blood flow in man. Teachers of physiology who have been striving to introduce medical students to the finer points of blood and gas analysis and the various techniques derived from the original Haldane analyzer and the VanSlyke apparatus now find their duties lightened through the widespread application of these principles in studies of the human circulation. Catheterization of the heart and other vessels, together with measurements of blood gas concentrations and of gas exchanges in the lungs, enable clinicians to decide whether the pathways of the circulation are normally arranged, whether by-passes exist (as in certain congenital anomalies), or whether some disease process extraneous to the circulatory system has brought about a redistribution of blood supply. Discoveries in this field have not only benefitted the patients concerned; they have strengthened teaching in physiology of the respiratory and circulatory systems.

Under the supervision of the Cardiovascular Study Unit these techniques are being applied in studies of the collateral circulation through lungs of patients with chronic pulmonary disease. The development of such collateral circulation and the channels through which it courses have been described by Liebow *et al.*,* together with experiments of a functional nature performed upon dogs. Gray and his associates are extending the observations to human patients, utilizing equations enabling them to calculate either the volume of the collateral circulation or the flow through non-aerated portions of the lung. Two methods are now in use for estimating these fractions. In the first method, through application of the Fick principle to the respiratory exchange, the minute output of the right heart may be determined; at the same time, by a rebreathing technique, the total amount of blood reaching ventilated alveoli may be estimated. If the latter quantity exceeds the former, it means that the alveoli must be perfused by blood not coming from the right heart—that is, by blood reaching the lungs through bronchial collaterals. But if the rebreathing method yields a lower flow than the Fick technique, it may be concluded that some of the blood pumped through the pulmonary circulation is not participating in a normal gas exchange. In the second method the fractional flows are calculated from mixing equations derived from the equations of Lundsgaard and VanSlyke.†

Employed in the diagnosis of congenital abnormalities of the great vessels, these principles have made possible numerous successful operations upon human patients. They are now being extended to studies of circulatory changes in chronic lung disease, in pulmonary arterial-venous fistulas, in myocardial infarction, and in combined heart and lung disease. Lurie, Whittemore, and Gray (in preparation) have recently noted that the accuracy of these methods may be somewhat less than it is usually supposed to be, and that one may sometimes be misled in diagnosis through physiological studies. Their findings can only mean that further research is needed in order to clarify the relations between physiological observations and disease processes of the circulatory and respiratory systems.

Operations upon the frontal lobes of the cerebrum. Within the past fifteen years a new surgical specialty known as "psychosurgery" has grown out of observations made upon two chimpanzees in the Laboratory of Physiology. Psychosurgery refers to operations upon the brain for the

* Liebow, A. A., Hales, M. R., Harrison, William, Bloomer, William, and Lindskog, G. E.: Yale J. Biol., 1950, *22*, 637.

† Lundsgaard, C. and Van Slyke, D. D.: Medicine, 1923, *2*, 1.

relief of mental illness. A number of different operations have been attempted, but all involve the interruption of connections of frontal cortex with other parts of the brain. Almost every form of serious mental illness has been treated by these operations, and the dramatic "cures" sometimes obtained have been widely publicized through newspapers and magazines.

The observations from which this therapy arose were made in 1935 by Carlyle Jacobsen in two chimpanzees, "Becky" and "Lucy," which were being used to test discriminatory functions of the frontal lobes.* The tests consisted of simple problems or puzzles which an animal can solve in order to obtain a bit of food. As is usual in such a program, the animals occasionally made incorrect choices and failed to earn their expected reward. To this situation they responded by temper tantrums—rolling on the floor, beating on the cage, defecation, and urination. It may be noted that the tantrums did not appear to be directed against the experimenter, but rather toward the animal itself. All of this behavior was altered following bilateral removal of certain portions of the frontal lobes, for although testing continued as before, the chimps were no longer disturbed when they made errors in judgment.

Following a report of these observations at the International Neurological Congress in London in 1935, a Portuguese neurosurgeon, Egas Moniz, devised the operation of leucotomy to interrupt frontal lobe projection fibers in human patients. For his contribution Professor Moniz was recently awarded the Nobel Prize (shared with Professor Hess of Zurich), and the use of operations of this type has spread world wide. Much has been learned in this way about frontal lobe function in man, although it is to be regretted that careful scientific study has not been possible in many instances. An attempt is now being made to correct this situation, however, not only here at Yale where the Connecticut Lobotomy Committee is conducting a co-operative study, but also in a number of other medical centers.

In view of the fact that the original observations were made upon animals, it is somewhat surprising to find that thousands of operations were performed upon human patients before further work was attempted in the laboratory. This situation is certainly to be contrasted with the caution and restrictions imposed in other fields of medicine—for example, in introducing new drugs, where thorough testing upon animals is required before use in humans is permitted. It is only within the past few years that adequate support has been provided for basic research upon the functions and connections affected by psychosurgery. The Veterans Administration now supports such a project in the Laboratory of Physiology. Here attention is focused upon "buried" regions of the cortex, the insula and areas adjacent to it, and the depths of the sulci where research has always been difficult. The orbital and medial surfaces of the hemispheres are likewise being studied, as is the temporal lobe. Many of these regions were formerly classed under the term "rhinencephalon,"† although it now appears that what was thought to be the primitive olfactory brain has an important place in patterns of behavior other than olfaction. Papers describing the results of this study are now appearing in the *Journal of Neurophysiology*, where they may serve as a stimulus to further progress in this vital field. It is reasonable to hope that our understanding of neurophysiology may progress at the same time that a greater insight is gained into the proper applications of psychosurgery.

* Fulton, J. F.: West. J. Surg., 1950, *58*, 246.
† MacLean, P. D.: Yale J. Biol., 1950, *22*, 437.

EVENTS

YALE MEDICAL SOCIETY

October 11, 1950

SEROLOGICAL EPIDEMIOLOGY WITH PARTICULAR ATTENTION TO POLIOMYELITIS ANTIBODIES IN VARIOUS PARTS OF THE WORLD. By John R. Paul and John T. Riordan.

Serum levels of various antibodies were studied in a relatively isolated part of the world where a high susceptibility to diseases of civilization might be expected. A settlement of Eskimos on the northern coast of Alaska was selected in view of its physical remoteness.

Two hundred and fifty blood samples were obtained from various age groups in the community. Although a number of antibodies, including gamma-globulin itself, were investigated, special attention was given to the specific neutralizing antibodies for the Lansing strain of poliomyelitis virus. The history of poliomyelitis in the district was difficult to obtain, but a report of two fatal cases in 1930 was viewed with some interest when the observations showed that antibodies were prominent in the group over eighteen years of age, and negligible in those younger. Antibodies and immunity are not necessarily correlated, but it was suggested that such studies be used as a "base line" for further work in epidemiology.

O. L. K.

CARBON MONOXIDE POISONING: SKIN LESIONS AND VASOMOTOR DISTURBANCES. By J. W. Meigs and J. P. W. Hughes.

With the object of determining the etiology of the skin lesions found in CO poisoning, a study was undertaken of all patients admitted to the New Haven Hospital since 1920 for CO poisoning. Certain findings seemed to be correlated with severe poisoning: elevation of temperature, respiration of 30/minute or over, elevated white cell count, lung signs, skin lesions, tendency to bleeding, pseudo-recovery, and albuminuria. Other signs, while not correlated with severity, seem involved in the disease: rapid pulse, abnormal blood pressure, abnormal skin color, cardiac arrhythmia, elevation of the red cell count, and glycosuria. The conclusion was that vasomotor disturbance is a likely predisposing factor to development of skin lesions, but that other factors (e.g., increasing anoxia) may also be present.

R. G.

THE FRAGMENTATION OF CALCULI BY ULTRA-SOUND. By Harold Lamport, H. F. Newman, and R. D. Eichhorn.

The basis of this work is the fact that, while solid material such as renal and biliary stones cannot withstand the high shearing stress of rapid vibration, fluid material such as soft tissue rebounds easily and is usually not disrupted by such stresses. Earlier work of this nature was carried out by Wood and Loomis with ice.

Lamport *et al.*, succeeded in fragmenting calculi under a variety of experimental conditions, e.g., submerged in oil or water in a cellophane bag or in a loop of intestine. The sound frequencies used were from 400 to 1200 kilocycles per second. Stones varied in responses from simple fracture

to complete suspension in the medium. Twenty per cent of stones were unaffected.

Lamport listed some of the potential difficulties in the work: the possibility of fragments acting as niduses for new calculi; possible damage to soft tissue, primarily from heat; slight hemolysis which has been reported; damage to bone lying close to the irradiated area; susceptibility of nervous tissue to radiation.

J. F. S.

HORMONAL ETIOLOGY OF OVARIAN AND LYMPHOID TUMORS IN IRRADIATED ANIMALS. By W. U. Gardner.

The hypothesis that ovarian and lymphoid tumors in roentgen irradiated mice are due to the resultant hormonal imbalance, rather than to a direct action of x-rays, was put forth.

Experimental work to substantiate this hypothesis may be summarized as follows: (1) Ovarian and lymphoid tumors were produced by irradiation of the bodies of mice. (2) Irradiation of one ovary produced ovarian tumors, but only if the non-irradiated ovary was removed. (3) The tumors were similar to those appearing in intrasplenic ovarian grafts in castrated animals in which a known hormonal imbalance exists. (4) Administration of estradiol benzoate, following irradiation of the bodies of mice, prevented the development of ovarian tumors. (5) Lymphoid tumors were also produced by irradiation and by estrogens. Administration of testosterone propionate decreased the number of tumors produced in both estrogen-treated and x-rayed mice. Estrogens, on the other hand, greatly enhanced the incidence of lymphomas in irradiated mice. The author did not extrapolate these results to account for spontaneous lymphoid tumors.

V. S. G.

PHARMACOLOGY SEMINAR

October 19, 1950

MECHANISM OF ANTI-FIBRILLATORY DRUG ACTION. By Joseph R. DePalma, M.D., Assistant Professor of Medicine, State University Medical Center at New York, College of Medicine.

In reviewing anti-fibrillatory drugs and their mechanism of action, the speaker described his recent investigations of alpha-fagarine, an alkaloid isolated in Argentina, and of a synthetic analogue, referred to as 1227. Both have greater anti-fibrillatory action than quinidine but demonstrate a similar property of inducing ventricular tachycardia by initiating ectopic foci. Quaternization of the compound 1227 resulted in a loss of activity, and, on the basis of this and other such observations, it was concluded that a tertiary amine rather than a quaternary amine is required to depress the auricles.

Evidence was presented that the seemingly contradictory effect of quinidine—depressing the auricles while stimulating the ventricles—could be ascribed to the drug's resulting in a greater increase of the refractory period of the auricle than of the ventricle. Electrocardiographic studies were conducted upon normal individuals and patients with chronic auricular fibrillation. Measurements were taken of the refractory period and contractility of isolated cat auricles and ventricles, the latter represented by

the papillary muscle. A method was described by which recordings could be made of ventricular threshold and contractility, concomitant with oscillographic strength-duration curves. By this means it was observed that acetylcholine will decrease chronaxie while increasing the rheobase of the papillary muscle.

Mecholyl, especially in patients with thyrotoxicosis, predisposes to ventricular tachycardia; dibenamine similarly lowered the threshold for ventricular tachycardia. Epinephrine, it was postulated, may cause auricular fibrillation by a vagal discharge which decreases the refractory period, a conclusion substantiated by observations of the effect of acetylcholine upon the isolated auricle and ventricle. A further postulation, that hydrocarbon anesthetics predispose to ventricular fibrillation by a decreased refractory period resulting from the negative inotropic activity of these agents, was presented. It was emphasized that tetany may occur in myocardial muscle when certain poisons interfere with the positive metabolic activity occurring during the refractory period.

In the discussion that followed, it was stressed that quinidine therapy is always contraindicated in digitalis intoxication.

V. S. G.

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BOOK REVIEWS

HEREDITY, EAST AND WEST. By Julian Huxley. New York, Henry Schuman, Inc., 1949. x + 246 pp. $3.00.

This account of the controversy occasioned by the new Lysenkoist "science" of genetics deserves at least a cursory reading by men in science, who must by and large not fully realize what has happened in Russia to the respectable science of genetics. Although somewhat wordy and repetitious, the book is well documented, providing convincing evidence that genetics in Russia has by state fiat become in fact a dogma.

The first chapter outlines the essentials of Neo-Mendelism as distinguished from Michurinism, the Russian system elaborated almost entirely by Lysenko and derived from Lamarckism. An historical account depicts the progressive fall from political favor of Neo-Mendelism and its proponents, dating back to 1932. The succeeding chapter on the "Ideological Issue" bears principally on the point of official Soviet condemnation of scientific results on other than scientific grounds, with a review of the politically weighted words attached to the systems in controversy, e.g., "Soviet, patriotic, materialist" as against "Western, bourgeois, idealist."

Following a discussion of the attitudes and methods of Lysenko, including a short description of the vernalization and grafting experiments and of Lysenko the man, there is an outline of modern Neo-Mendelism and Neo-Darwinism, together with important supporting evidence. This latter chapter is written primarily for the layman.

The fifth, and central, chapter, entitled "Totalitarian Regimentation of Thought," scans the effects of such regimentation in Soviet Russia in politics, social science, philosophy, law, arts and music, and science, where even relativity is assailed as bourgeois and idealist! Why has the sociopolitical struggle been transferred into genetics? Among several reasons put forward the most important appear to be: first, a desire for easy intelligibility to the masses and quick, practical agricultural results, and secondly, ideological inacceptability of human inability to change nature at will and more specifically of any innate inequalities among human individuals.

In his last chapter, in which Professor Huxley discusses what can be done, he understandably falls down. He views the Russian situation as an exaggeration of the general trend toward greater centralization and organization of society. The desideratum is the preservation of scientific autonomy of thought and freedom of communication in the face of inevitably increasing governmental support and direction of scientific programs. His suggested solutions—the adoption of a universal ideological aim, such as "evolutionary humanism," and the issuance of manifestoes by scientific societies stating the rights and responsibilities of scientists—seem hardly adequate. A final point is well taken, however, that widespread familiarity with the events in Russia may serve to alert scientists elsewhere to the dangers of Lysenkoism at home.

W. McN.

AN ATLAS OF HUMAN ANATOMY. By Barry J. Anson. Philadelphia and London, W. B. Saunders Company, 1950. xxi + 518 pp. $11.50.

This fine atlas comprises the results of the studies and skill of Professor Anson, many of his students, and five artists. Much of the content has

previously appeared in journals. In examining the several hundred illustrations in the book, it is refreshing to find a succession of diagrams, line drawings, and halftones prepared according to various techniques. The subtle and harmonious contrasts to be observed offer a pleasant change from the conspicuously unvarying style that one sometimes finds in a large collection of illustrations prepared by a single individual. The pictures, often in color, are clear, vigorous, and not too elaborate. An impression of depth and texture is given without excessive use of shading. The labels, almost entirely in Latin, are beautifully executed.

The utility and professional excellence of the dissections, and of their interpretation, by Dr. Anson and his colleagues and pupils continue a high standard. The dissections are realistic rather than idealized, are arranged on a regional basis, and are often progressive. Most pages include at least a few lines of textual explanation and comment. Due attention has been given to the portrayal of anatomical variations. This reviewer particularly enjoyed two pages of diagrams and text explaining some of the terminology applied to muscles.

The table of contents (more than 12 pages) and index (14 pages) are sufficiently detailed and are organized both regionally and alphabetically. Format, printing, paper, and binding are excellent.

This book will be useful to students, teachers, and practitioners of medicine.

THOMAS R. FORBES

THE NATURE OF THE BACTERIAL SURFACE. Edited by A. A. Miles and N. W. Pirie. Springfield, Ill., Charles C Thomas, 1950. vii + 179 pp. $3.00.

This volume is a record of the proceedings of a symposium held by the Society for General Microbiology in London in April, 1949. The papers, nine in number, are reproduced in full together with discussions. The contributions are chiefly British, though there is representation from American, French, and Netherlands laboratories.

Topics covered include investigation of bacterial surfaces by antigenic analysis, biochemical methods, osmotic studies, and adsorption of bacteriophage. Other papers deal with capsule formation in the pneumococcus and the question of the relation of flagella to motility. Included with the papers are twelve excellent plates made from electron micrographs.

These papers are highly detailed and technical. While of considerable interest to the microbiologist, this book is rather too specialized for more than a cursory glance by one whose interests are chiefly clinical.

W. McN.

BIOMETRICAL GENETICS. By K. Mather. New York, Dover Publications, Inc., 1949. ix + 158 pp. $3.50.

In a small book intended only for the advanced student or worker in the field, Dr. Mather has given a rather neat, though admittedly incomplete, coverage of the statistics of continuous variation based on a theory of summation of polygenes, which is the field of biometrical genetics. The historical and philosophical development of biometrical genetics is briefly reviewed. Next, the determination of a suitable metric scale, in the absence of adequate theoretical considerations, is covered. Then, probably the most important result of biometrical genetics is discussed: the partition of variation into fixable and unfixable (according as they are utilizable in the

selection of true breeding strains) heritable variation, and non-heritable (environmental) variation. Finally, the effects and tests of linkage, the estimation of the number of effective factors, and the speed of advance under selection are considered.

Many of the formulae presented are clearly derived from fairly elementary statistical considerations, and related formulae are usually nicely tabled. All methods of analysis are cogently presented, and suggestions as to the future development of methods are given. The limitations of all individual methods, of biometrical genetics in general, and of this book in particular are all well stated by the author, something very necessary to the user of unfamiliar statistical method. This book is written for a limited audience, but will probably be highly welcomed by that audience.

R. G.

THE MERCK MANUAL, 8TH EDITION. Rahway, N. J., Merck and Company, 1950. 1570 pp. $5.00.

The first seven editions have proved the worthiness of this pocketsized compendium of medical information. The eighth edition, completely rewritten, is not likely to be any less well received than its predecessors, for in its many, full-packed, thin sliced pages there is veritable encyclopedic coverage of clinical and laboratory topics. Much new material has been added which will increase the usefulness of this volume. As before, there are sections on diseases and major symptoms with the emphasis on diagnosis and therapy. It is in the latter that the editors have erred on the side of dogmatism, for in those conditions in which a considerable difference exists in regard to therapeutic regimes, only one plan is given and the controversies not indicated (a case in point is the discussion of the management of diabetic coma). In appropriate conditions the pathology and physiology are adequately discussed to provide the necessary background for rational therapy. A helpful feature is the thumb index which allows one to turn immediately to the several sections. There are many cross-references and a complete index. Prescriptions are included at the end of each section with proprietary preparations plainly labeled as such. Alternate and commercially competitive products are also listed. For those wishing a portable medical library, *The Merck Manual* comes closest to what the doctor ordered.

W. F. S.

AN INTRODUCTION TO THE STUDY OF EXPERIMENTAL MEDICINE. Claude Bernard. New York, Henry Schuman, Inc., 1949. $3.00.

The rapid gains of experimental biology more than adequately document the efficacy of the scientific method. There were investigators before Bernard who reasoned in a similar manner and planned experiments accordingly, but it was Bernard who crystallized the concept and proceeded to demonstrate by his own investigations the wealth of information which could be gained by methodical "questioning" of the living organism. It was his opinion that life is based on physico-chemical phenomena and that eventually living and non-living would be found to obey the same physico-chemical laws. The relationships of parts of the living organism are more complex than those of the non-living and demand that the complex phenomena of life be dissociated into more and more simple phenomena which are amenable to analysis. The method of analysis is always the same: the hypothesis is formulated; the interrelated phenomena of life are altered

in such a way as to test the hypothesis; the observations gained from the experiment are incorporated in a new hypothesis. Bernard recognized that the success or failure of the procedure was in great part dependent upon the attitude of the investigator and demanded open-mindedness, doubt, and unprejudiced observation.

Today we recognize that investigation of the properties of living material can not be carried out in any other manner than that which Claude Bernard advocated. It is difficult for us to realize how far ahead of the medicine of his day he was and how much his experiments and writings contributed to the understanding of the organism. Bernard's style is simple and lucid, so much so in fact that his misconceptions (as in his condemnation of statistical methods) are made as obvious as his truths. One cannot help marvelling at the freshness of this book, written nearly a century ago by the man who founded modern physiology. His reasoning is as valid as it ever was; his method, although nowhere definitely set down as a system, is the only one. *An Introduction to the Study of Experimental Medicine* should be read and studied by all persons who would consider themselves educated.

In contrast with its content, the physical character of this edition is such as to indicate a definitely limited span of life. The covers are thin and susceptible to bending at the corners without great provocation. The type is frequently blurry and the intensity of printing often varies from page to page.

R. R., JR.

PROGRESS IN CLINICAL ENDOCRINOLOGY. S. Soskin, Editor. New York, Grune and Stratton, 1950. 622 pp. $10.00.

In a period marked by unprecedented advance in clinical endocrinology, this volume of critical reviews in each phase of the subject offers the student, practitioner, and specialist alike a composite, meaningful picture of the field. In addition to discussions of each endocrine organ, there are specialized topics entitled: "Special Aspects of Sex Endocrinology"; "Use of Hormones in Non-Endocrine Conditions," including a chapter on the use of ACTH and cortisone in rheumatoid arthritis and other conditions; "General Endocrine Topics"; and an excellent introductory section comprising the chapters, "The Role of Hormones in Metabolism" by Drs. Soskin and Levine, and "Endocrine Regulation of Protein Metabolism" by Dr. Abraham White.

Ninety-one authorities have contributed a total of seventy-eight articles. The authors present summaries of the facts from the literature with the original investigators' interpretations. Although specialists may not concur with all the conclusions of the authors, the presentation is both factual and contemplative. Represented among the contributors to the eleven chapters on the adrenal cortex are Drs. Sayers, Ingle, and Thorn. The sections on the thyroid, adrenal medulla, pancreas, ovary, testis, and the pituitary are again indicative of the editor's discriminating choice of authors. Among the articles on the thyroid gland is a provocative review of the evidence against the "iodine-lack" theory of the etiology of endemic goiter.

The book is as up to date as the exigencies of publishing allow. There are numerous references to papers published in 1949. The attractively bound volume is adequately indexed and contains a satisfactory number of charts,

graphs, and illustrations. No typographical errors were apparent to this reviewer.

In editing a volume of such scope, it is necessary for the editor to be arbitrary in the selection of topics and the extent to which each is covered. Dr. Soskin has wisely delegated eighty-five pages to diabetes mellitus, but he may well have extended the twelve pages on the adrenal medulla to include a more detailed description of therapy in hypertensive states and to illumine more clearly the status of epinephrine and nor-epinephrine. On the other hand, the chapter on antithyroid drugs has been judiciously limited to those agents which are of clinical efficacy rather than including the numerous drugs which have been tested.

The stated objective of the editor is to develop the newer and borderline aspects of the subject into an organized pattern. The objective was more than adequately fulfilled.

V. S. G.

MULTI-ENZYME SYSTEMS. By Malcolm Dixon. New York, Cambridge University Press, 1949. 100 pp. $1.75.

This little book presents four special lectures given by Dr. Dixon at University College, London, in May, 1948. As always in his work, it furnishes, first, a basis for the better understanding of the subject, and next, a fine stimulus to thought and study. The lectures begin with the statements that enzymes are the working parts of living cells, and that, for the cell to achieve all of the metabolic transformations of which it is capable, not single enzymes, but systems of enzymes are involved and must be objects of study. These statements are illustrated by a brief discussion of the special properties of enzymes and by reference in detail to the system of anaerobic glycolysis and to the oxidation system of the "tricarboxylic acid cycle." The second lecture is devoted to the systems of hydrogen transfer and of phosphate transfer. It is shown how the one is designed to realize the energy of various substrates and how the other is designed to accept such energy, in the convenient general form of the "high-energy phosphate bond," and to distribute it among the many pathways through which the work of the cell is done.

In the third lecture Dr. Dixon discusses the oxidation-reduction systems of the cell and develops in some detail the concept of the "rH" scale, originally suggested by Mansfield Clark in 1923. Students who have wrestled with descriptions of oxidation-reduction potentials may find enlightenment in this discussion and in the 10 simple rules governing "rH." In the last lecture applications of "rH" are illustrated, and the same general idea is applied to the systems of phosphate transfer, relating the different substrates concerned to their "phosphate-pressure," analogous to the hydrogen pressure in reducing systems. An "rP" chart is constructed from the limited available data, and a simple set of rules, similar to those governing rH, is developed and presented. The concept promises to be immensely useful in bringing into orderly relationship the growing body of information about the organic compounds of phosphorus involved in the metabolism of the cell. The book is written in a clear and simple style which makes it deceptively easy to read, and an undiminished pleasure to re-read in study.

ALFRED E. WILHELMI

WORLD SURGERY, 1950. By Stephen A. Zieman. Philadelphia, J. B. Lippincott Company, 1950. 161 pp. $6.00.

This book is a collection of abstracts of the world literature in surgery written by the abstract and news editor of the *Journal of the International College of Surgeons*. The volume is attractive in format and is easy to use. Complete references are listed at the end of each abstracted article, most of which were published in 1949. The papers were chosen on the basis of the unusual, the interesting, and the new in the theory and practice of surgery. For the surgeon who has scant time to thumb through his journals, this volume should be of help in keeping up with advances in fields other than his own and in learning of the achievements of his foreign colleagues.

W. F. S.

THE ABC OF ACID-BASE CHEMISTRY. By H. W. Davenport. Chicago, University of Chicago Press, 3d edition, 1950. 86 pp. $2.00.

Davenport's classic monograph appears for the third time with several notable improvements. The binding, though paper, is more attractive and the size is smaller and less cumbersome. The author and printer have achieved clarity both in technical matters, such as the printing of formulas, and in the minor revisions of the text. In general, there are no drastic reversals of theory, although "dissolved carbonic acid" is spoken of as "dissolved carbondioxide" in the new edition, and the method recommended for the determination of alkali reserve is changed. Perhaps the most important additions are a bibliography and seven new sections. These include: methods for laboratory analyses and general discussions of (1) carriage of oxygen in the blood, (2) buffers, (3) chemical regulation of respiration. There is also expansion of the section on renal compensation. Departures from the 2d edition, then, are for the most part in the direction of clarification, and those who find the subject confusing should certainly acquire the new edition.

O. L. K.

SIR WILLIAM OSLER: APHORISMS FROM HIS BEDSIDE TEACHINGS AND WRITINGS. Collected by Robert Bennett Bean. Edited by William Bennett Bean. New York, Henry Schuman, Inc., 1950. 159 pp. $2.50.

While on rounds with Osler during 1903-05, R. B. Bean kept notes of the more pithy of Osler's spontaneous remarks while in the wards. From these notes, Dr. Bean's son, W. B. Bean, has compiled and edited this little volume of 357 Osler aphorisms, his purpose being "to introduce him (Osler) to a new generation of medical students, and to refresh the memory of an older generation." The result is a delightful collection of epigrams which not only serves these purposes, but stands in its own right as another independent view, in this sense neither introduction nor refresher, of a fascinating personality. Through the charm and humor of Osler's own words, we glimpse the many facets of his character: the Spartan nature of both his professional and private life, his devotion and unselfishness toward his students, his ideas on education and examinations, his advice to students (including many pointers on clinical observation and technique), his philosophy concerning life and his profession, and his deep love for his work. This

collection seems certain to serve its function of refreshing the memory of the older generation of medical students. For the new generation, a volume of individual aphorisms cannot, of course, be more than a brief introduction, but a more stimulating and entertaining introduction than this volume would be difficult to find.

R. G.

PRACTICAL GYNECOLOGY. By W. J. Reich and M. J. Nechtow. Philadelphia, J. B. Lippincott Company, 1950. 426 pp. $10.00.

Two Cook County Hospital staff members have prepared a text for the non-specialist on the subject of "everyday" gynecology. Their efforts have given us an extremely readable and worthwhile volume. The entire book could be read in a few evenings' concentration. It is chatty in style but serious in content. The book is liberally illustrated and contains a unit of fifteen plates of exceptionally fine colored photographs of various pathological conditions. The authors explain clearly the simple methods and practical procedures used in obtaining diagnoses by the physician in office practice. Students are sure to appreciate this volume, for it gives a complete coverage of the field but does not bog down in the rare, the esoteric, or the finely detailed discussions of more standard texts.

W. F. S.

MR. CARLYLE MY PATIENT. By James L. Halliday. New York, Grune & Stratton, 1950. 227 pp. $3.50.

Thomas Carlyle, Victorian man of letters, lived for eighty-six years. For sixty-three of those years he suffered from pains in the upper abdomen which he called "dyspepsia" and from chronic constipation, depression and irritability which he termed "biliousness." Author Halliday, from a scholarly study of Carlyle's works, letters, pamphlets, and journals, has pieced together a searching psychobiography of the gaunt, brusque Scotsman. Readers will be interested especially in the treatment of the legend of Carlyle's alleged impotence. The fact that the author's subject was a voluble and prolific writer with a psychological "orientation" has given this study advantages over other latter-day analyses of historical figures. The work is presented as a case study with the appropriate Carlylean quotations, arranged in chronological reference, interpreted and explained in language more or less intelligible to the psychiatrically uninitiated. Continuity is provided by conventional biographical methods. This volume should provide exciting tea time talk for Carlyle's twentieth century disciples and admirers but a somewhat less surprised reception from psychiatrists who are sure to have heard this story, with modifications, repeated often by other voices in other rooms.

W.F.S.

A HISTORY OF BIOLOGY. By Charles Singer. New York, Henry Schuman, Inc., 1950. xxv + 579 pp. $5.

Charles Singer has made a distinguished contribution to the Life of Science Library series. In this book, which was previously printed in America under the title *The Story of Living Things,* he presents in chronological order the inception and progress of the fundamental ideas upon which the science of Biology stands.

By sacrificing minute detail in order to preserve continuity, he writes a concise and lucid account of the important observations, concepts, and men of biology. Singer gives the reader a rounded, understandable background for each idea as it takes its place in the story. The book will gratify those readers who want a full view of the important steps in the science without the confusion of small detail and blind alleys.

J.F.S.